Parasite Immunology, 2000: **22:** 259–263

Evidence for genomic imprinting of the major QTL controlling susceptibility to trypanosomiasis in mice

STEVEN J.CLAPCOTT[1], ALAN J.TEALE[2] & STEPHEN J.KEMP[1]

[1]School of Biological Sciences, Donnan Laboratories, University of Liverpool, Liverpool L69 7ZD, UK and
[2]International Livestock Research Institute, PO BOX 30709 Nairobi, Kenya

SUMMARY

Inbred strains of laboratory mice exhibit marked differences in survival time following infection with Trypanosoma congolense*, the principal cause of trypanosomiasis in African livestock. The difference in survival time between the relatively resistant C57BL/6 J and more susceptible BALB/c inbred strains has been attributed to three quantitative trait loci (QTLs),* Tir1, Tir2 *and* Tir3. *In order to determine whether there was a parent-of-origin effect on this trait, four backcross populations derived from the C57BL/6 J and BALB/c parental strains were bred and inoculated with* T. congolense. *The two populations with F_1 fathers and BALB/c mothers had a significantly greater overall survival rate than the two populations with BALB/c fathers and F_1 mothers. This pattern of inheritance suggested the involvement of imprinted genes. Genotyping with markers at the three QTLs controlling susceptibility revealed that the difference in survival time was consistent with genomic imprinting of the QTL of largest effect,* Tir1.

Keywords *genomic imprinting, mouse, QTL, susceptibility, trypanosomiasis*

INTRODUCTION

Tsetse fly transmitted trypanosomes (*Trypanosoma* spp.) cause 'sleeping sickness' in man, and have a serious impact on livestock farming in large areas of Africa. The principal livestock-infective trypanosome species*, Trypanosoma congolense*, is readily transmitted to laboratory mice. The C57BL/6 J (B6) inbred strain is relatively resistant to trypanosomiasis following *T. congolense* challenge, having a mean survival time of 110·2 days, while the BALB/c (C) strain is more susceptible, having a mean survival time of 49·5 days (Morrison *et al*. 1978). The resistance of the C57BL/6 J strain is controlled by three quantitative trait loci (QTLs), *Tir1*, *Tir2* and *Tir3*, positioned on chromosomes 17, 5 and 1 with 95% confidence intervals (CI) of 0·9 cM, 12 cM and 10 cM, respectively (Kemp *et al*. 1996, 1997, Iraqi *et al*. 2000).

Genomic imprinting is a process through which the expression of a gene is dependent on the sex of the parent from which it was inherited, the repressed allele generally considered to be the imprinted one (John & Surani 1996). Although parent-of-origin effects were first recorded by mule breeders in Asia Minor > 3000 years ago (Morison & Reeve 1998), formal demonstration of genomic imprinting was not achieved until 1991, when the selective maternal expression of *Igf2r*, the paternal expression of *Igf2* and the maternal expression of *H19* in mice were reported (Barlow *et al*. 1991, Bartolomei *et al*. 1991, De Chiara *et al*. 1991). Subsequently, 35 genes in the mouse have been shown to be subject to genomic imprinting (Beechey *et al*. 1999). In addition, several phenotypes in humans, mice and other animals have been recognized which show a pattern of inheritance consistent with the involvement of imprinted genes (Morison & Reeve 1998).

On the basis of the proximity of *Tir1* to known imprinted regions of chromosome 17 (Beechey *et al*. 1999), we monitored survival time following *T. congolense* challenge in four independent backcross (BC) populations (Table 1) to determine if there was an imprinting effect on this trait.

Correspondence: S.J.Clapcott, Department of Zoology, University of Oxford, Oxford OX1 3PS, UK
Received: 23 September 1999
Accepted for publication: 8 February 2000

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Table 1 Construction of backcross populations

Backcross	Resistant genome origin	Resistant parent[a]	MtDNA origin[b]	Sex chromosomes[c]	
				Male	Female
(B6CF1)C[d]	Female	Mother	B6	$X^{C \text{ or } B6} + Y^{C}$	$X^{C \text{ or } B6} + X^{C}$
C(B6CF1)[e]	Female	Father	C	$X^{C} + Y^{C}$	$X^{C} + X^{B6}$
(CB6F1)C[f]	Male	Mother	C	$X^{C \text{ or } B6} + Y^{C}$	$X^{C \text{ or } B6} + X^{C}$
C(CB6F1)[g]	Male	Father	C	$X^{C} + Y^{B6}$	$X^{C} + X^{C}$

[a]Assumed to be heterozygous (F_1) parent carrying resistant (B6) alleles. [b]Mitochondrial origin assumed from ancestry. [c]Strain of origin of the X- and Y-chromosomes. [d](B6CF1)C: B6 mat. grandmother, C mat. grandfather, C pat. grandmother, C pat. grandfather. [e]C(B6CF1): C mat. grandmother, C mat. grandfather, B6 pat. grandmother, C pat. grandfather. [f](CB6F1)C: C mat. grandmother, B6 mat. grandfather, C pat. grandmother, C pat. grandfather. [g]C(CB6F1): C mat. grandmother, C mat. grandfather, C pat. grandmother, B6 pat. grandfather.

Mice from each population were later genotyped at simple sequence length polymorphisms (SSLPs) within the 95% confidence intervals (CIs) of *Tir1*, *Tir2* and *Tir3*. This approach revealed that mice inheriting the resistance (B6) allele of *Tir1* from their father had a higher rate of recovery from trypanosomiasis compared to those inheriting the same allele from their mother.

MATERIALS AND METHODS

Mice

Parental strains of mice were BALB/c OlaHsd (C) and C57BL/6 J OlaHsd (B6) (Harlan UK Ltd, Bicester, Oxon, UK). CB6F1 and B6CF1 mice of both sexes were backcrossed to the susceptible (BALB/c) parental strain to generate the following BC populations (Table 1): (B6CF1)C, C(B6CF1) (CB6F1)C and C(CB6F1).

T. congolense challenge

At 10–12 weeks of age, 60 males and 60 females from each BC population, together with 20 males and 20 females from both parental strains, were challenged with *T. congolense* clone 1180 (Masake *et al*. 1983) by intraperitoneal inoculation of 4×10^4 (200 μl) bloodstream-form parasites. Parasitaemia were confirmed by thick blood film examination in a total of 560 mice, while a total of 59 were excluded due to failure to demonstrate established parasitaemia. To monitor any background death rate, five males and five females of both parental strains were not infected. The experiment was terminated 175 days post inoculation.

DNA analysis

From each BC population, 15 mice with the shortest survival times, 15 with the longest survival times, and 10 with mid-range survival times were genotyped at SSLPs *D1Mit91*, *D1Mit102* and *D1Mit105* on chromosome 1, *D5Mit200*, *D5Mit114* and *D5Nds4* on chromosome 5, and *D17Mit80*, *D17Mit16* and *D17Mit177* on chromosome 17. *D17Mit16*, *D5Mit114* and *D1Mit102* are known to occur within the 95% CI of *Tir1*, *Tir2* and *Tir3*, respectively (Iraqi *et al*. 2000). Genomic DNA was prepared from tail tissue by salt/ethanol precipitation. Primers for polymerase chain reaction (PCR) amplification were obtained from Research Genetics (Huntsville, AL, USA). Primers were labelled with ^{33}P, and used to amplify SSLPs according to the supplier's recommendations. PCR products were separated by polyacrylamide gel electrophoresis, and visualized with a phosphor imaging system.

Statistical analysis

Kaplan–Meier survival probabilities for each BC population were calculated from the numbers of mice alive at daily intervals following *T. congolense* inoculation (Altman & Bland 1998, Bland & Altman 1998). The survival probabilities for each BC population were plotted, and the log-rank method was used to test for differences in overall survival (Peto 1974).

Linkage analysis

Association between the SSLP alleles at each *Tir* QTL and survival time was measured using MAPMAKER/QTL (Lander *et al*. 1987). Based on the overall survival rates following *T. congolense* challenge (Figure 1), BC populations with F_1 mothers ($F_1 \times C$) were combined and analysed separately from BC populations with F_1 fathers ($C \times F_1$). Genetic linkage maps for chromosomes 17, 5 and 1 were based on SSLP positions given in the Mouse Genome Database (Mouse Genome Database 1998). Survival time data from all mice, whether genotyped or not, were used in

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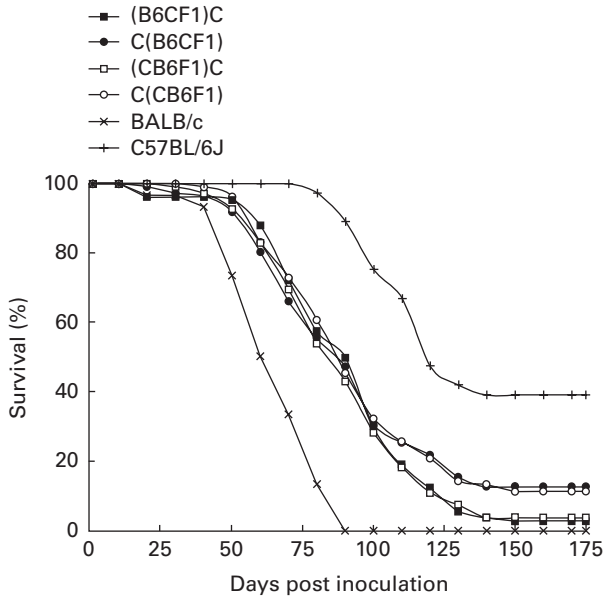

Figure 1 Survival of BALB/c and C57BL/6J parental strains and four backcross populations following *T. congolense* inoculation.

the analysis. BC mice still alive at the end of the challenge were given an assumed survival time of 142 days, one day after the last BC mouse died.

RESULTS

Survival following *T. congolense* challenge

The four BC populations had similar mean survival times following *T. congolense* challenge, ranging from 86·04 to 91·19 days. In comparison, the mean survival time of the C57BL/6J parental strain was 119·81 days, which is almost twice that of the BALB/c parental strain (61·27 days).

The BC populations had roughly intermediate rates of survival compared with the parental strains (Figure 1). All four BC populations had very similar survival rates until approximately 100 days post inoculation, with there being little difference between them in time to 75% survival (64–69 days), 50% survival (84–89 days) and 25% survival (104–112 days). After approximately 100 days post inoculation (B6CF1)C and (CB6F1)C had equivalent survival rates, as did C(B6CF1) and C(CB6F1), which were higher. At the termination of the experiment, 175 days post inoculation, four (B6CF1)C (3·7%), four (CB6F1)C (3·6%), 14 C(B6CF1) (12·6%), and 12 C(CB6F1) (11·3%) were still alive and had no detectable parasitaemia. The rate of recovery from trypanosomiasis was thus three-fold higher for the populations with F_1 fathers than for the populations with F_1 mothers.

Log-rank tests revealed no significant difference in overall survival between (B6CF1)C and (CB6F1)C (chi-squared = 0·118; d.f. = 1; $P = 0{\cdot}73$), and between C(B6CF1) and C(CB6F1) (chi-squared = 0·0343; d.f. = 1; $P = 0{\cdot}85$). There was, however, a significant difference in overall survival between populations with F_1 fathers and BALB/c mothers [C(B6CF1) and C(CB6F1)] and those with F_1 mothers and BALB/c fathers [(B6CF1)C and (CB6F1)C] (chi-squared = 4·17; d.f. = 1; $P = 0{\cdot}041$).

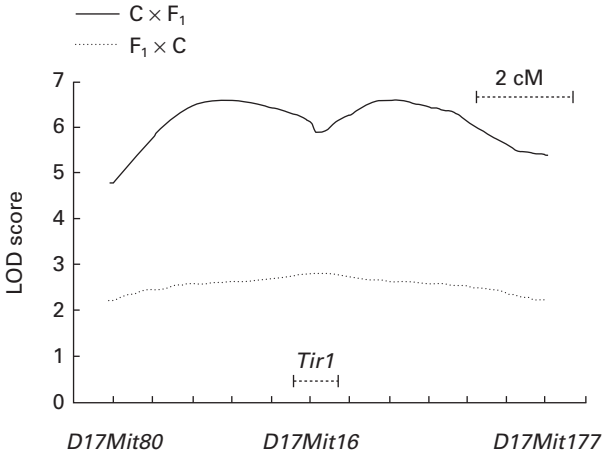

Figure 2 LOD scores associated with SSLP markers across the 95% confidence interval of *Tir1* on mouse chromosome 17 for $C \times F_1$ and $F_1 \times C$ backcross populations.

Linkage analysis

Linkage analysis with MAPMAKER/QTL revealed a marked difference in LOD score on chromosome 17 between the $F_1 \times C$ [(B6CF1)C and (CB6F1)C] and $C \times F_1$ [C(B6CF1) and C(CB6F1)] populations. At the SSLP within the 95% CI of *Tir1*, *D17Mit16*, the $F_1 \times C$ populations had a LOD score of 2·7 while the $C \times F_1$ populations had a LOD score of 5·9 (Figure 2). The higher LOD score of the $C \times F_1$ populations is consistent with the higher overall survival rates of these populations. The $C \times F_1$ populations also had a higher LOD score on chromosome 1 than the $F_1 \times C$ populations, but the difference was much smaller. (B6CF1)C and (CB6F1)C had a LOD score of 2·2 at the SSLP within the 95% CI of *Tir3*, *D1Mit102*, while that of C(B6CF1) and C(CB6F1) was 2·8. *Tir3* on chromosome 5 was not detected in this study; no BC population had a LOD score greater than 0·3, indicating that there was no association with survival time on this chromosome.

DISCUSSION

Four BC populations (B6CF1)C, C(B6CF1) (CB6F1)C, and C(CB6F1), were inoculated with *T. congolense*. After approximately 100 days, BC populations (B6CF1)C and

(CB6F1)C had equivalent survival rates, as did BC populations C(B6CF1) and C(CB6F1), which were higher. At the termination of the experiment, 175 days post inoculation, the number of survivors from the C(B6CF1) and C(CB6F1) BC populations was over three times greater than the number of (B6CF1)C and (CB6F1)C survivors. BC populations with F_1 fathers thus had a significantly higher overall survival rate following inoculation with *T. congolense* than BC populations with F_1 mothers.

This parent-of-origin effect could not be explained by mitochondrial DNA (mtDNA) polymorphism because there was no difference in survival rate between BC populations (B6CF1)C and (CB6F1)C, which had mtDNA of C57BL/6 J origin and BALB/c origin, respectively (Table 1). This finding is supported by the results of a study of mtDNA sequences from various mouse populations, in which no variation was found between the common laboratory strains of inbred mice, including BALB/c and C57BL/6 J (Ferris *et al*. 1982).

There was no male-specific or female-specific difference in survival rate between C(B6CF1) and C(CB6F1), which differed in the strain origin of their sex chromosomes, indicating that the parent-of-origin effect could not be attributed to an X- or Y-chromosome polymorphism. The BALB/c and C57BL/6 J strains both have Y-chromosomes of *Mus m. musculus* origin, whilst some other laboratory strains, such as AKR/J and SWR/J, have the *M. m. domesticus* type (Nishioka 1987).

The significant difference in overall survival between C(B6CF1) and (CB6F1)C, which differed in the zygosity of their mothers but had mtDNA and Y-chromosomes of the same BALB/c origin, leaves genomic imprinting as the most likely explanation for the parent-of-origin effect on overall survival following *T. congolense* inoculation. Linkage analysis showed that association between survival time and SSLP alleles at *Tir1* was approximately 100-fold greater in the progeny of BALB/c mothers and F_1 fathers than in the progeny of F_1 mothers and BALB/c fathers. This result is consistent with the differential expression of *Tir1* alleles depending on the sex of the parent from which they were inherited. Although we believe this to be the first demonstration of an imprinting effect on resistance to infection in mice, linkage to *Tir1* does not necessarily imply that this QTL is directly responsible for the parent-of-origin effect on survival.

The 95% CI of *Tir1*, at 17·4–18·3 cM from the centromere (Iraqi *et al*. 2000), is not in a known imprinted region but is flanked by regions of chromosome 17 that are known to be imprinted (Beechey *et al*. 1999). The paternally imprinted insulin-like growth factor 2 receptor (*Igf2r*) and the maternally imprinted insulin-like growth factor 2 receptor antisense (*Igf2ras*) (Beechey *et al*. 1999) have been mapped to 7·35 cM from the centromere (Mouse Genome Database 1998), which is 10 cM centromeric of *Tir1*. Telomeric of *Tir1*, paternal disomy of chromosome 17 distal of the T138Ca translocation breakpoint causes small body size (0·7 × normal) evident from day 7 after birth (Beechey *et al*. 1999).

Although inheritance of the B6 allele conferred a greater survival time following *T. congolense* inoculation, it is currently impossible to determine whether *Tir1* is maternally or paternally imprinted because it is not known whether expression of the underlying gene is beneficial or detrimental to survival. If the B6 allele confers expression of the underlying gene, the greater survival time of mice that inherit it from their father suggests maternal imprinting of *Tir1*. Conversely, if the B6 allele confers repression of the gene, paternal imprinting is more likely.

The strongest evidence for the presence of genomic imprinting is direct detection of parent-of-origin-specific transcription from a gene, but it will not be possible to demonstrate this for *Tir1* until the underlying gene has been identified. Regions of differential DNA methylation mark all imprinted genes studied to date. Whereas most of these genes are methylated on the repressed allele some, such as *Igf2r*, *Igf2* and *Snrpn*, also contain methylation on the expressed allele (Reik & Walter 1998). The original pattern of cytosine methylation is erased in cloned sources of DNA (Boehm 1998), so it will not be possible to identify methylated regions within a BAC or PAC clone physical map of *Tir1*. The putative imprinting of *Tir1*, however, might have an important bearing on the evaluation of candidate genes for the QTL; those genes with monoallelic expression being stronger candidates than biallelically expressed genes.

ACKNOWLEDGEMENTS

Robert King, of the International Livestock Research Institute (ILRI), Nairobi, developed and managed the mouse populations used in this study. Henrie Gathuo and Moses Ogugo, of ILRI, provided technical support. John Rowlands, of ILRI, and David Papworth of the MRC Mammalian Genetics Unit, Harwell, provided expert advice on statistical analysis. Chris Graham of the Department of Zoology, University of Oxford, and two anonymous referees provided useful comments on the manuscript. This work was supported by a Project Grant from the Wellcome Trust (045410/Z/95/Z) to S.J.K. and a Quota Research Studentship from the Biotechnology and Biological Sciences Research Council (BBSRC) to S.J.C.

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