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Research Article

Genetic basis of the resistance to *Strongyloides venezuelensis* (Nematoda, Rhabdiasidae) infection in mice (*Mus musculus*)

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Abstract

We investigated the resistance to *Strongyloides venezuelensis* primary infection of mice strains NIH (resistant) and C57BL/6 (susceptible) and the F1 and F2 offspring of crosses between these strains. The mice were infected with 2000 larvae and seven days later were sacrificed for parasite recovery and counting. There was no statistically significant ($p > 0.05$) sex effect on resistance. The F1 mice showed an intermediate mean number of parasites as compared to the parental NIH and C57BL6 strains. Out of 400 F2 mice, the 10% most resistant mice were infected with 21 to 97 parasites, while the 10% most susceptible mice were infected with 1027 to 1433 parasites. We also found that F2 mice with black fur (n = 72), the same color as the C57BL/6 susceptible parental strain, were more susceptible than white (n = 104) or gray furred (n = 224) mice. It is conceivable that some genes determining coat color are located on the same chromosome as where genes controlling helminth resistance.

Key words: mice, *Strongyloides venezuelensis*, resistance, genetics.

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Introduction

In animal production, parasitic infections are an important factor which increase production costs, principally due to reduced yield and, in the case of helminth infections, the cost of anthelmintics. Helminth control in domestic animals is mainly based on treatment with anthelmintics, but this practice is becoming less effective due to the development of resistant parasite populations (Amarante *et al.*, 1992; Echevarria *et al.*, 1996; Waller, 1997).

Genome mapping techniques have been employed to identify molecular markers associated with genes for economically important traits or disease resistance, allowing the selection of more productive or resistant animals (Gogolin-Ewens *et al.*, 1990). It is assumed that many yield-related traits in livestock and plants are controlled by a large number of genes from different loci called quantitative trait loci (QTL) (Georges, 2001). Therefore, a QTL may comprise many genes that account for host protection against pathogens (Valladares-Hernandez *et al.*, 2004).

Mice (*Mus musculus*) are one of the main experimental models used in genetic mapping for the identification of molecular markers associated with resistance to parasitic infections. Complex resistance mechanisms can be easily analyzed through controlled crosses between resistant and susceptible inbred strains of mice (Marshall *et al.*, 1992; Darvasi, 1998). Mice have been used as experimental model because they are simple, fast and relatively cheap to raise and maintain in large numbers.

Molecular markers associated with resistance to nematode infection in mice may contribute to mapping orthologues in ruminants because of the high chromosomal and gene similarity observed in mammals from comparative genome mapping data of ruminants, mice and humans (Lyons *et al.*, 1997; Modi *et al.*, 1998; O'Brien *et al.*, 1999; Amarante *et al.*, 2000; Band *et al.*, 2000; Iannuzzi *et al.*, 2001; Amarante and Amarante, 2003). It is important to remember that comparative maps have also been built to identify QTLs and eligible genes acting as a source of molecular markers for marker assisted selection (Rexroad *et al*., 2001).

Studies on acquired resistance to gastrointestinal nematodes in mice using crosses between resistant and susceptible mice strains have shown that a large number of QTLs are involved in the immunological response of the hosts (Behnke *et al.*, 2003; Iraqi *et al.*, 2003; Menge *et al.*, 2003). However, there is also innate resistance against parasitic infections, which is observed in some strains of mice

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after primary infection by helminths. This is the case of the NIH strain of mice that proved to be highly resistant to primary infection by *Strongyloides venezuelensis* (Nematoda: Rhabdiasidae) in comparison with the C57BL/6 mouse strain (Amarante and Oliveira-Sequeira, 2002). *Rattus norvegicus* is the natural host of S. *venezuelensis* (Nakai and Amarante, 2001) but this nematode also easily infects mice, which are thus extensively used as models in studies of the immunology of nematode diseases (Negrão-Corrêa *et al.*, 2004; Sasaki *et al.*, 2005).

The broad aim of the research described in this paper is to elucidate the complex interaction between *Strongyloides* infections and the host response. We report on a study which compared the *S. venezuelensis* primary infection response of NIH (resistant) and C57BL/6 (susceptible) mice strains and the F1 and F2 generation of crosses between these strains. The association between sex, coat color and resistance to *S. venezuelensis* infection was also evaluated.

Material and Methods

Animals and artificial infection

We obtained *Mus musculus* mice strains NIH from Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil and C57BL/6 from the Vivarium Center (Centro de Bioterismo), Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil. Male and female mice and their descendants were kept at the Laboratory of Animal Experimentation, Departamento de Parasitologia, Instituto de Biociências, UNESP-Botucatu, Brazil.

The *Strongyloides venezuelensis* which we used was originally obtained from wild rats (*R. norvegicus*) at the beginning of the 1980s and has been kept in Wistar rats (*R. norvegicus*) at the Departamento de Parasitologia, UNESP, Botucatu.

Infective *S. venezuelensis* larvae in the L_3 stage were produced by mixing feces from infected rats with sterilized equine feces, humidifying the mixture and incubating it in a Petri dish at 25 °C for three days. After incubation the L_3 larvae were recovered by filtering and decanted into distilled water using a Baermann apparatus. The number of larvae in 10 µL samples of the larval suspension were counted, using ten replicates, and the number adjusted to a suitable concentration for use in the experiments. We infected five-week to seven-week old mice by injecting them subcutaneously with 0.1 mL of the larval suspension containing 2000 *S. venezuelensis* larvae (Amarante and Oliveira-Sequeira, 2002).

Parental and F1 susceptibility (experiment I)

We mated seven NIH females and six C57BL/6 females with males of their own strains, resulting in 58 offspring for the NIH strain and 48 for the C57BL/6 strain. We also mated six NIH females with six C57BL/6 males to produce 33 F1 offspring and we also mated four C57BL/6 females with four NIH males to produce 40 offspring, the total F1 generation for this set of matings therefore being 73. The resistance was evaluated in groups of five males and five females randomly chosen from the offspring as follows: NIH pure-bred; C57BL/6 pure-bred; F1 hybrids from the NIH male x C57BL/6 female cross; and F1 hybrids from the C57BL/6 male x NIH female cross. The mice were infected described above and then humanely sacrificed seven days later to determine the *S. venezuelensis* burden.

F2 susceptibility (experiment II)

In this experiment we mated three NIH females with three C57BL/6 males and four C57BL/6 females with four NIH males to produce 19 F1 siblings which were crossed to yield 400 F2 mice (210 males, 190 females). The five-week to seven-week old F2 animals were infected with 2000 *S. venezuelensis* larvae and sacrificed as described. Tail samples of each animal were stored at –80 °C for subsequent DNA extraction.

Worm burden determination

It has been shown that the highest number of parthenogenetic *S. venezuelensis* females is detected seven days after infection (Oliveira-Sequeira and Amarante, 2001; Nakai and Amarante, 2001) and that more than 95% of the parasites in mice are located in the first 14 cm of the small intestine, which, on average, is less than one-third of the length of the small intestine (Nakai and Amarante, 2001). Based on these considerations, we sacrificed the mice seven days after infection and dissected out the initial third of the intestine from each mouse, sliced the excised intestine longitudinally and attached it to a wire support which we placed in a 20 mL tube in such a way as to prevent the intestine touching the bottom of the tube. Sufficient aqueous saline solution (0.85% NaCl) was added to cover the intestine and the tubes incubated at 37 °C for four hours, after which the intestines were shaken vigorously to completely release the nematodes and then formaldehyde solution (38% w/w formalin) was added at a final concentration of 5% (v/v) and the parasites counted using stereoscopic microscope.

Statistical analysis

The data from experiment I were analyzed using two classes (sex and group) and the PROC general linear model (GLM) of the SAS® 2001 program version 8. In experiment II, the statistical analysis was similar, but the classes were sex and coat color. Means were compared by the minimum significant difference at the 5% of probability level.

The statistical analysis was performed with transformed data (Log_{10}) but, for clarity, the non-transformed arithmetic means (± standard error, SE) or the data frequency are given in the results section.

Results

Parental and F1 susceptibility (experiment I)

The sex of the mice had no influence on the number of parasites recovered ($p > 0.05$). The mean small intestine *S. venezuelensis* burden of the F1 mice from the NIH male x C57BL/6 female cross was 303.9 ± 43.5 while that of the C57BL/6 male x NIH female cross was 229.0 ± 34.8, these values being not significantly different at $p > 0.05$.

Figure 1 shows the number of *S. venezuelensis* specimens recovered from the small intestines of the parental strains and the F1 crosses. The degree of resistance of the F1 mice was intermediate compared to that of the parental strains of the same age.

F2 susceptibility (experiment II)

As with experiment 1, the sex of the mice did not influence parasite number. The mean small intestine *S. venezuelensis* burden for the 210 males was 539 ± 26.6 while that of the 190 F2 females was 513 ± 24.8 ($p > 0.05$).

The mean small intestine *S. venezuelensis* burden was 624 ± 41.3 for black F2 mice (n = 72), 507 ± 35.3 for white F2 mice (n = 104) and 504 ± 24.6 for gray F2 mice (n = 224). Black F2 mice showed a significantly higher ($p < 0.05$) parasite burden than white or gray mice.

Figure 2 shows the frequency distribution of *S. venezuelensis* recovered from the small intestines of infected F2 mice. The group of the 10% most resistant mice showed 21 to 97 parasites, while the group of the 10% most susceptible mice showed 1027 to 1433 parasites. The F2 mice used in this experiment were the descendants of seven NIH and C57BL/6 couples, but there was no influence of these couples on the susceptibility of the F2 animals ($p > 0.05$).

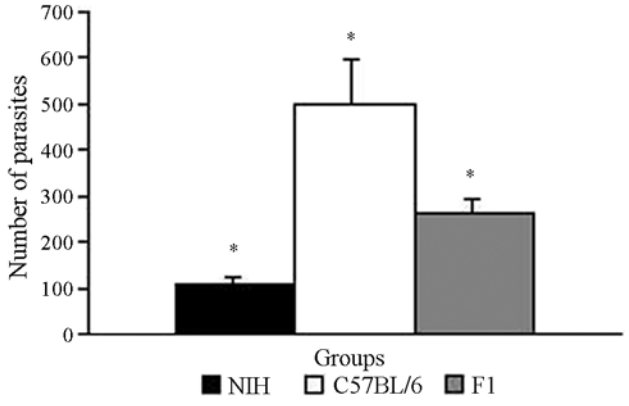

Figure 1 - Number of *S. venezuelensis* parthenogenetic females recovered from the first third of the small intestines of NIH, C57BL/6 and F1 mice infected with 2000 larvae. (*) Significantly different, $p < 0.05$ (PROC GLM- SAS®, 2001, version 8). Vertical bars indicate the standard error.

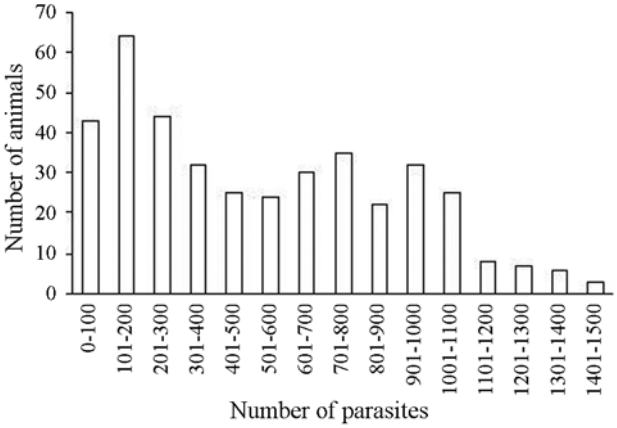

Figure 2 - Frequency distribution of the number of *S. venezuelensis* recovered from the small intestines of F2 mice seven days after infection with 2000 larvae.

Discussion

The NIH mice were more resistant to *S. venezuelensis* infection than the C57BL/6 mice, which agrees with the findings of Amarante and Oliveira-Sequeira (2002). The F1 mice showed an intermediate parasite burden in comparison to both the resistant (NIH) and susceptible (C57BL/6) parental strains. However, different results have been reported for NIH x CBA (susceptible) F1 mice infected with *Trichuris muris* (Wakelin 1975) and NIH x B10 (susceptible) F1 mice infected with *Trichinella spiralis* (Wakelin, 1980), for which the parasite burden in F1 animals was similar to that found in the resistant parental strain. According to Wakelin (1980), the low response of the susceptible strain to *T. spiralis* infection is inherited as a recessive trait. Behnke and Robinson (1985) demonstrated that F1 mice (NIH x C57BL/10 or NIH x B10G) showed a better response than the resistant NIH parental strain when infected by *Nematospiroides dubius*.

Amarante *et al.* (1999b) observed that adult F1 sheep from the cross between Florida Native (resistant) x Rambouillet (susceptible) were resistant to natural infections caused by gastrointestinal nematodes. However, lambs artificially infected with *Haemonchus contortus* were susceptible and showed similar performance to the susceptible breed (Amarante *et al.*, 1999a). Together, these results suggest that there is a high level of heterosis which is favorable to gastrointestinal nematode resistance, although there are some exceptions as witnessed by the results published by Amarante *et al.* (1999a) and those shown in the present paper.

It has been reported that the average number of the gastrointestinal nematode *Heligmosomoides polygyrus* in F2 mice was closer to that found in the resistant SWR strain than in the susceptible CBA strain (Iraqi *et al.*, 2003). However, our experiments with *S. venezuelensis* showed high variability in the F2 mice, with many siblings being highly resistant while others were highly susceptible.

Nakanishi *et al*. (1989) demonstrated that testosterone reduced the response of lymphocytes, macrophages and eosinophils to primary infection of C57BL/6 mice by *Brugia pahagi*, consequently reducing resistance. Rivero *et al*. (2002) observed that, due to reduced plasma testosterone, castrated Wistar rats were less susceptible to *S. venezuelensis* primary infection in comparison to non-castrated rats and also found a significant increase in the susceptibility to *S. venezuelensis* in castrated Wistar females treated with testosterone. However, contrasting with these reports, our study indicated that the sex of the mice had no influence on the *S. venezuelensis* burden.

In our study, curiously, F2 mice with a black coat, which is the color of the susceptible parental strain (C57BL/6), were more susceptible than white or gray mice. Jordan and Beermann (2000) state that 90 loci are known to influence coat color in mice, and it is possible that genes determining coat color are located on the same chromosome as genes responsible for resistance or susceptibility to helminth infection.

It is important to point out that in our experiments with *S. venezuelensis* we investigated the resistance of the host under primary infection, because it has been reported that both the NIH and C57BL/6 strains showed strong immunity to secondary infections by *S. venezuelensis* (Amarante and Oliveira-Sequeira, 2002). In contrast, Iraqi *et al.* (2003) investigated the acquired resistance of mice after several infections with *H. polygyrus* and found seven QTLs related to acquired resistance on six chromosomes of F2 mice produced by crossing the resistant SWR strain with the susceptible CBA strain. The data and material obtained in our the present study will be used to identify QTLs associated to innate resistance to *S. venezuelensis* infection and may clarify the situation.

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