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Tolerance and resistance to a nematode challenge are not always mutually exclusive

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ABSTRACT

The relationship between the manifestations of tolerance (a host's ability to reduce the impact of a given level of pathogens) and resistance (a host's ability to clear pathogens) has been assumed to be an antagonistic one. Here we tested the hypothesis that mice from strains more resistant to intestinal nematodes will experience reduced tolerance compared with less resistant mice. Three inbred strains of mice were used: C57BL/6 mice have been characterised as susceptible, whereas BALB/c and NIH mice have been characterised as resistant to *Heligmosomoides bakeri* infection. Mice of each strain were either parasitised with a single dose of 250 L_3 *H. bakeri* ($n = 10$) in water or were sham-infected with water ($n = 10$). Body weight, food intake and worm egg output were recorded regularly throughout the experiment. Forty-two days p.i. mice were euthanised and organ weights, eggs in colon and worm counts were determined. C57BL/6 mice showed significantly greater worm egg output ($P < 0.001$), eggs in colon ($P < 0.05$) and female worm fecundity ($P < 0.05$) compared with NIH and BALB/c mice. Parasitised BALB/c mice grew more whilst parasitised C57BL/6 mice grew less than their sham-infected counterparts during the first 2 weeks post-challenge ($P = 0.05$). Parasitism significantly increased liver, spleen, small intestine and caecum weights ($P < 0.001$) but reduced carcass weight ($P < 0.01$). Average daily weight gain and worm numbers were positively correlated in NIH mice ($P = 0.05$); however, the relationship was reversed when carcass weight was used as a measure for tolerance. BALB/c mice did not appear to suffer from the consequences of parasitism, with carcass weight similar in all animals. Our hypothesis that strains more resistant to the *H. bakeri* infection are less tolerant compared with less resistant strains is rejected, as the two resistant strains showed variable tolerance. Thus, tolerance and resistance to an intestinal nematode infection are not always mutually exclusive.

1. Introduction

The intestinal trichostrongyloid nematode *Heligmosomoides (polygyrus) bakeri* has been used as a model of chronic intestinal nematode infection for over four decades (Behnke et al., 2006). *Heligmosomoides bakeri* infection in mice induces a strongly polarised Th2 immune response, which has been shown to be critical for worm control and expulsion (Reynolds et al., 2012). The mechanisms underpinning helminth expulsion in mice are studied to facilitate predictions about the outcome of similar interactions between helminths and the immune system in livestock and humans, to enable the development of control strategies. The outcome of primary *H. bakeri* infection is strongly influenced by the genetic backgrounds of mice, with strains differing in their susceptibility to chronic infection (Reynolds et al., 2012). Whilst between-strain variation in nematode resistance has been previously described (Behnke et al., 2006), there is no evidence of description of variation in tolerance. Resistance describes the ability of the host to clear pathogens, whereas tolerance describes the ability to reduce the health or fitness impact of a given infection intensity (Ayres and Schneider, 2012; Raberg, 2014). Characterising the tolerance of mouse strains that differ in their resistance to *H. bakeri* infection will facilitate the selection of the most appropriate mouse strain to model nematodiasis in human and livestock hosts.

The study of tolerance to parasites and its association with resistance has a long tradition in plant science but very limited evidence

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is available from animals. Recently, the individual variation in tolerance to parasites has been described in wild sheep and rodent populations (Hayward et al., 2014; Jackson et al., 2014). Hayward et al. (2014) observed a positive relationship between tolerance and evolutionary fitness in sheep, with more tolerant animals having higher lifetime breeding success. Jackson et al. (2014) on the other hand observed a negative relationship between tolerance and reproduction, with more tolerant animals having reduced reproductive effort. The association between the manifestations of tolerance and resistance to nematodes in animals has been previously assumed to be an antagonistic one (Doeschl-Wilson et al., 2009), although there is no experimental evidence to support this view. Råberg (2014) argued that when analysed simultaneously, tolerance and resistance should be under correlational selection; this implies either a negative relationship between these traits or the possibility they may both be at intermediate levels.

Here we tested the hypothesis that between-strain variation in resistance to *H. bakeri* infection will correlate with between-strain variation in tolerance. The expectation was that mouse strains more resistant to *H. bakeri* infection will be less tolerant compared with less resistant strains.

2. Materials and methods

2.1. Experimental animals and housing

The animal experiments were approved by the Scotland's Rural College (SRUC), UK, Ethical Review Committee (ED AE 06/2011) and carried out under Home Office authorization (PPL 60/3626). A total of 60 5 weeks old male C57BL/6, NIH and BALB/c mice ($n = 20$ per strain), were housed in a room with an ambient temperature of 21 ± 1 °C and a 12:12 h light:dark cycle (light cycle: 07:00–19:00 h). Mice were individually housed in solid bottomed cages with fresh sawdust and bedding material provided weekly. Shredded paper was added as environmental enrichment. The three strains were selected based on variation in their susceptibility to infection with *H. bakeri* as defined by their phenotype during the infection. C57BL/6 mice have been characterised as poor responders to *H. bakeri*; they maintain a high worm burden that can persist for over 30 weeks (Behnke et al., 2006). NIH mice have been characterised as strong, early responders to *H. bakeri*; compared with C57BL/6 mice, worm establishment is lower and worm burdens are cleared within 7 weeks (Behnke et al., 2006). BALB/c mice have also been characterised as strong responders, although the worm expulsion rate is slower than in NIH mice (Behnke et al., 2006; Reynolds et al., 2012).

2.2. Infection protocol and experimental design

The experiment was conducted over two consecutive blocks, balanced for all treatments, of 30 mice each (60 mice in total, 20 from each strain). At day 0 of each block, mice of each strain (C57BL/6, NIH and BALB/c) received either a single dose of 250 *H. bakeri* L_3 suspended in 0.2 ml of water ($n = 10$ per strain) or a sham infection of 0.2 ml of water ($n = 10$ per strain) via oral gavage (Houdijk and Bünger, 2007). The *H. bakeri*, formerly known as *H. polygyrus bakeri* and *Nematospiroides dubius* (Cable et al., 2006), were cultured from mono-specifically-infected donor mice. The dose of *H. bakeri* was chosen to produce a subclinical level of infection that has been shown to affect mouse growth (Houdijk and Bünger, 2006, 2007).

Mice were fed ad libitum throughout the experiment with a maintenance diet (14% crude protein; Special Diet Services, Lillico Biotechnologies, UK). Mice were monitored for 42 days p.i. On day 42 they were euthanised for sample recovery.

2.3. Measurements and sample collection

2.3.1. Body weight (BW) and food intake

Mice and food refusals were weighed three times weekly throughout the experiment. On each of these days food refusals were weighed out and fresh food added was weighed in. From these measurements, food intake was calculated per mouse per day. Food intake and BW of mice were used as indicators of growth performance.

2.3.2. Nematode egg counts

Mice were placed onto wire-bottomed cages and faecal samples were collected on wetted cardboard on days 17, 24, 31, 38 p.i. to assess faecal egg counts (eggs per g of faeces). This was carried out using a modified flotation technique (Christie and Jackson, 1982). Faeces were collected over a 12 h period, during which a constant rate of egg production was assumed. Egg output (EO) was expressed as eggs per 12 h to account for a possible dilution effect on faecal egg counts attributed to variable faecal outputs as a consequence of different food intake by different mouse strains (Coltherd et al., 2009).

2.3.3. Internal organ weights, eggs in colon and worm burdens

On day 42 mice were humanely killed via CO_2 inhalation and dissected for sample recovery. The small intestine was weighed, opened and placed in a tube with PBS, which was then incubated at 37 °C for 3 h to allow worms to migrate out of the tissue. Tissue and recovered worms were stored in a 5% formalin solution. Male and female worms were separated and counted. The colon contents were weighed and an egg count was performed using the same floatation technique described in Section 2.3.2 (Christie and Jackson, 1982). The colon egg count was then multiplied by the weight of colon contents to account for dilution effects arising from variation in food intake and colon content volumes between the different strains. Resultant data were expressed as the number of eggs in colon (EIC). The EIC was divided by the number of female worms counted to obtain an estimate for the *per capita* fecundity (eggs per female). EO, EIC, *per capita* fecundity and total worm counts were used to confirm variation in resistance of the mouse strains. EIC and worm counts were used in tolerance estimates, as explained in Section 2.3.4.

2.3.4. Measures of tolerance

Individual tolerance was estimated in two different ways. Firstly, we associated carcass weight at dissection (true reflection of performance) and worm burdens recovered at dissection (accurate estimate of parasite load). We also associated average daily weight gain, which is often used as an indirect indicator for performance, and EIC as an indirect indicator for parasite load. Our expectation was that tolerance estimates would be similar in both cases.

2.4. Statistical analysis

Data were analysed in Genstat 11th Edition (VSN International LTD, 2008). Model assumptions were tested on normality of means and residuals. Average daily BW gain and food intake during worm establishment (Period 1 (P1): days 0–16) and the established infection (Period 2 (P2): days 17–42) were analysed through a 3×2 factorial ANOVA (three strains × two levels of parasitism) with pre-infection BW used as a covariate. Carcass and internal organ weights were analysed through the same 3×2 factorial ANOVA, again with pre-infection BW as a covariate. EIC, EO, worm counts and worm fecundity were analysed in parasitised animals by one-way ANOVA (three strains). The EO data were analysed in a repeated measures model. Due to the skewed nature of the data,

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