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Response of the *Strongyloides ratti* transcriptome to host immunological environment

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ABSTRACT

The immunological environment experienced by parasitic nematodes varies greatly between hosts and is particularly influenced by whether or not a host has been previously infected. How a parasitic nematode responds to these different environments is poorly understood, but may allow a parasite to ameliorate the adverse effects of host immunity on parasite fitness. Here we use a microarray approach to identify genes in the parasitic nematode *Strongyloides ratti* that exhibit differential transcription between different rat host immunological environments, and between replicate lines of *S. ratti* selected for either early or late reproduction. We hypothesise that such genes may be used by this species to cope with and respond to its host environment. Our results showed that, despite large phenotypic differences between *S. ratti* adults from different immunological environments, the *S. ratti* transcriptome exhibited a relatively stable pattern of expression. Thus, differential expression amongst treatments was limited to a small proportion of transcripts and generally involved only modest fold changes. These transcripts included a group of collagen genes up-regulated in parasites early in an infection, and in immunised host environments, which may be related to protection against the damage caused to a parasite by host immune responses. We found that later in an infection, a number of genes associated with muscle function and repair were up-regulated in immunised host environments; these may help parasites maintain their position in the host intestine. Differences in transcription between selection lines of *S. ratti* were only observed in immunised hosts and included genes associated with the response to the host's immunological environment.

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1. Introduction

Little is known about how parasitic nematodes respond to their host's immunological environment (Thompson et al., 2008). The immunological responses made by hosts to infection act to reduce the fitness of parasitic nematodes (Anderson and May, 1992; Paterson and Viney, 2002; Bleay et al., 2007) and induce increasingly adverse effects on parasite fitness during the course of an infection as the host immune response develops (Wakelin, 1996; MacDonald et al., 2002; Wilkes et al., 2004, 2007). The immunological environment experienced by a parasitic nematode therefore varies substantially during the course of an infection. Furthermore, the immunological environments experienced by parasitic nematodes will vary between different individual hosts within natural populations due to variation in the innate susceptibility of different hosts, or due to variation in previous infection histories between hosts (Faulkner et al., 2002; Grenfell et al., 2002; Wakelin et al., 2002).

Given that the immunological environments of hosts are highly variable, one might expect parasitic nematodes to exhibit physiological or biochemical responses that are induced by the host's immunological environment. Thus, whilst a parasite might not escape the adverse effects of host immune responses entirely, the ability of a parasite to respond to its immunological environment may at least ameliorate their adverse effects. But alternatively, parasitic nematodes may be subject to molecular or physiological constraints that limit their ability to respond to the host's immunological environment (van Straalen and Roelofs, 2006; Thompson et al., 2008).

Several studies have reported functional variation in life-history traits between different parasitic nematode genotypes. Examples include variation in establishment, survival or reproduction within their host (Pozio et al., 1992; Bellaby et al., 1995; Paterson and Viney, 2003). In part, such variation may be mediated by the ability of different parasitic nematode genotypes to cope with their host's

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immune response. In support of this, we have found that life-history traits of a nematode parasite, *Strongyloides ratti*, respond to artificial selection. Specifically, worms selected to survive late into an infection ('Slow' lines) were better able to reproduce in a 'high' acquired immunity environment found late in an infection, and in a high con-specific density (which is correlated with levels of acquired immunity), than worms selected for reproduction early in an infection ('Fast' lines) (Paterson and Barber, 2007).

Here we investigated the transcriptional response of different *S. ratti* genotypes to the host's immunological environment. We found an overall pattern of relatively stable transcription amongst immunological environments and between genotypes. However some genes, particularly collagen genes and those involved in muscle repair, exhibited differences in transcription between worms from previously exposed and naive hosts and between Fast- and Slow-selected genotypes. These may contribute to the fitness differences between these genotypes in host environments with a high level of acquired immunity.

2. Materials and methods

2.1. Parasites and experimental design

Strongyloides ratti is a nematode parasite of rats (*Rattus norvegicus*) commonly used as a laboratory model for nematode infections (Dawkins, 1989; Viney, 1999). Natural infection is by skin penetration of infective L3s (iL3s), followed by tissue migration to the small intestine (Tindall and Wilson, 1988, 1990) and the development of parasitic females by day 4 p.i. (Kimura et al., 1999). By day 5 p.i., parasitic females produce eggs by mitotic parthenogenesis, which are passed in the faeces and can develop either directly into iL3s (homogonic development) or via free-living males and females that mate and produce eggs, which then develop into iL3s (heterogonic development) (Harvey et al., 2000). The parasite lines used are described in Paterson and Barber (2007). Briefly, selection lines were generated by a cross between two geographically distinct parasite genotypes (ED248 and ED321), infection of 100 iL3s into each of a pair of female Wistar rats (Charles River, UK) followed by either: (i) a 'Fast' selection regime, where eggs were collected from faeces 5 days p.i., or (ii) a 'Slow' selection regime, where eggs were collected at least 34 days p.i. Selection lines were maintained continuously in this manner for at least 40 generations. An additional line, LIV4, was maintained following a cross between ED248 and ED321 from eggs collected randomly between days 5 and 34 p.i. and used to provide iL3s for primary (immunising) infections.

Two experiments were performed (Table 1 and Fig. 1). Experiment 1 consisted of a total of 24 female Wistar rats. Groups of 12 animals were given either a primary infection of 10 LIV4 iL3s or a PBS sham infection. All animals were treated on day 22 p.i. with 12.25 mg of thiabendazole (Sigma, UK) to remove the primary infection. On day 29 p.i., groups of six animals from each primary infection treatment were given a challenge infection of 1000 iL3s from either $LIV2_S$ or $LIV12_F$ (Slow and Fast lines, respectively). Groups of three animals from each treatment combination were culled on days 34 and 41 p.i. (equivalent to days 5 and 12 post challenge infection (p.c.i.)) and parasitic females collected and stored in Trizol as described in Evans et al. (2008). Experiment 2 consisted of a total of 48 female Wistar rats. Groups of 16 animals were given a primary infection of either 10 LIV4 iL3s, 20 LIV4 iL3s or a PBS sham infection and then treated with thiabendazole on day 22 p.i. as above. On days 29 and 31 p.i., animals received challenge infections of 500 iL3s from one of four Fast lines ($LIV10_F$, $LIV12_F$, $LIV13_F$ or $LIV14_F$) or one of four Slow lines ($LIV2_S$, $LIV3_S$, $LIV5_S$ or $LIV6_S$). Two consecutive infections of 500 iL3s, rather than a single infection of 1000 iL3s, were used to attempt to reduce the variability in parasite establishment inherent from single dose infections. Faeces were collected on day 40 p.i. and cultured to measure the output of viable eggs. Animals were culled on day 42 p.i. and parasitic females collected as above. To reduce the number of samples handled on any single day, this experiment was split into eight blocks of six animals, such that each block consisted of three animals infected with a single Fast line and three animals infected with a single Slow line. Different combinations of Fast and Slow lines were used in each block. All animal procedures were conducted under UK Home Office licence and with approval from the University of Liverpool Animal Welfare Committee.

2.2. Microarray analyses

RNA was extracted from parasitic females, amplified and labelled with Cy3 and Cy5 dyes by the procedure described in Evans et al. (2008) except that amplification was performed using a MessageAmp™ II aRNA Amplification Kit (Ambion, Texas, USA) for Experiment 1 and a TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0 (Epicentre Biotechnologies, Wisconsin, USA)

Table 1
Summary of experimental infections with *Strongyloides ratti* in this study.

Number of animals	Primary infection Day 0	Clear infection Day 22	Challenge infection Day 29 (+31 for Exp. 2)	Sample parasites
Experiment 1				
3	PBS	Thiabendazole	1000 $LIV12_F$ iL3s	Day 34 (Day 5 p.c.i.)
3	PBS	Thiabendazole	1000 $LIV2_S$ iL3s	Day 41 (Day 12 p.c.i.)
3	PBS	Thiabendazole	1000 $LIV12_F$ iL3s	Day 34 (Day 5 p.c.i.)
3	PBS	Thiabendazole	1000 $LIV2_S$ iL3s	Day 41 (Day 12 p.c.i.)
3	10 LIV4 iL3s	Thiabendazole	1000 $LIV12_F$ iL3s	Day 34 (Day 5 p.c.i.)
3	10 LIV4 iL3s	Thiabendazole	1000 $LIV2_S$ iL3s	Day 41 (Day 12 p.c.i.)
3	10 LIV4 iL3s	Thiabendazole	1000 $LIV12_F$ iL3s	Day 34 (Day 5 p.c.i.)
3	10 LIV4 iL3s	Thiabendazole	1000 $LIV2_S$ iL3s	Day 41 (Day 12 p.c.i.)
Experiment 2				
8	PBS	Thiabendazole	500 + 500 Fast line iL3s[a]	Day 42 (c. Day 12 p.c.i.[b])
8	PBS	Thiabendazole	500 + 500 Slow line iL3s[a]	Day 42 (c. Day 12 p.c.i.[b])
8	10 LIV4 iL3s	Thiabendazole	500 + 500 Fast line iL3s[a]	Day 42 (c. Day 12 p.c.i.[b])
8	10 LIV4 iL3s	Thiabendazole	500 + 500 Slow line iL3s[a]	Day 42 (c. Day 12 p.c.i.[b])
8	20 LIV4 iL3s	Thiabendazole	500 + 500 Fast line iL3s[a]	Day 42 (c. Day 12 p.c.i.[b])
8	20 LIV4 iL3s	Thiabendazole	500 + 500 Slow line iL3s[a]	Day 42 (c. Day 12 p.c.i.[b])

Exp., experiment; iL3s, infective L3s; p.c.i., post challenge infection.
[a] Each animal was infected with one of four Fast selection lines or one of four Slow selection lines.
[b] Parasitic females were a mixture of 11 and 13 day old individuals.

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