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Genes important in the parasitic life of the nematode *Strongyloides ratti*

Fiona J. Thompson *, Gary L.A. Barker, Louise Hughes, Mark E. Viney

School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK

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Abstract

Parasitic nematodes are important pathogens of humans and other animals. The genus *Strongyloides* has both a parasitic and a free-living adult generation. *S. ratti* infections of its rat host are negatively affected by the host immune response, such that a month after infection, worms are lost from the hosts. Here we have investigated the changes in parasite gene expression that occur as the anti-*S. ratti* immune pressure increases.

Existing *S. ratti* expressed sequence tags were used to construct a microarray consisting of 2227 putative genes. This was probed with cDNA prepared from parasites subject to low or high immune pressures. There are significant changes in the gene expression of *S. ratti* when subject to different immune pressures. Most of the genes whose expression changes have no significant alignment to known genes. These data together with previous *S. ratti* EST data were then used to identify genes that we hypothesise are central to the parasitic life of *S. ratti* and, perhaps, other parasitic nematodes. These analyses have identified genes likely to play a key role in the parasitic life of *S. ratti*; these genes should be the priority for further investigation.

Keywords: *Strongyloides ratti*; Gene expression; Microarrays; Parasitic nematodes

1. Introduction

Parasitic nematodes infect approximately a quarter of the world's human population, with this concentrated in the developing world, especially those in poverty. Nematode parasitism is also universal in domestic and wild animals.

Nematodes of the genus *Strongyloides* infect a wide variety of terrestrial vertebrate hosts, and there are two species that infect humans, *S. stercoralis* and *S. fuelleborni*. It is estimated that some 50–100 [1] or 200 [2] million people are infected worldwide. The life cycle, unusually, includes a facultative dioecious free-living adult and an obligate female-only parasitic generation. For the well-studied species *Strongyloides ratti*, a parasite of the rat, parasitic females lie embedded in the mucosa of the small intestine of the host. These females reproduce by mitotic parthenogenesis and lay eggs that pass with the host faeces into the environment, where the development of the free-living stages occurs. Here both direct development to infective third stage larvae (iL3) or indirect development into free-living adult males and females occurs, with this developmental 'choice' controlled both by conditions external to the host as well as the immune status of the host from which eggs were passed [3].

Rats infected with *S. ratti* generate an effective anti-*S. ratti* immune response which ultimately eliminates the infection after approximately a month [4]. The anti-*S. ratti* immune response is a T-helper 2 (Th2)-type response, particularly characterised by the production of the cytokine interleukin 4. Such a Th2-type response is in common with immune responses generated against many parasitic nematodes [5]. For primary *S. ratti* infections of rats, the response consists of specific anti-*S. ratti* circulating IgG_1 and intestinal IgA responses and an intestinal mast cell response [6–8]. During this immune response, *S. ratti* parasitic females shorten in length and their *per capita* fecundity is reduced; the parasitic females also adopt a more posterior position in the host intestine [9]. These effects are reversible if the host is immunosuppressed [10]. Further, these effects do not occur in infections of nude rats [9,11] and, in contrast, in such animals *S. ratti* parasitic females live for a year, ultimately dying due to senescence [12].

As the rat host mounts this anti-*S. ratti* immune response and brings about these deleterious effects on the parasitic females, it is unlikely that they are passive partners. Thus, it can be

Abbreviations: iL3, infective third stage larvae; L1, first stage larvae; p.i., post-infection; RT-PCR, reverse transcriptase polymerase chain reaction; ESTs, expressed sequence tags; Hyp, hypothetical; ORF, open reading frame.

* Corresponding author. Tel.: +44 117 928 8111; fax: +44 117 331 7985.
E-mail address: F.Thompson@bristol.ac.uk (F.J. Thompson).

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envisaged that the parasitic females will seek homeostasis as their surrounding environment changes and this, in itself, is likely to have other consequences for the worms. Indeed, in observing the changes that occur during an infection, what is 'cause' and what is 'effect' is not clear [13]. For example, is the more posterior intestinal position of parasitic females [9] (i) a protective response, perhaps moving away from inflammation in the anterior part of the gut or (ii) is it a sign that the worms' health and fitness is being reduced such that they can no longer maintain an anterior position?

Recently there have been extensive expressed sequence tag (EST) gene discovery programmes for many species of parasitic nematodes, including *S. ratti* and *S. stercoralis* [14–16]. An analysis of the nematode EST data have shown that there are significant gene differences between different nematode species and groups. For example, an average of 45% of predicted genes from each nematode species appear to be unique to the phylum and 23% of putative genes are unique to the species from which they were found [15,17].

For *S. ratti*, EST analysis has identified 4251 clusters (*i.e.* likely genes) from both the free-living and parasitic stages of *S. ratti* [6]. Approximately a quarter of these likely genes have no significant alignment to genes known from other organisms. This high level of difference is consistent with what has been observed for many other nematode species [15,17]. Significant life-cycle stage-specific representation of these EST clusters was also observed [16,18]. Analysis of the EST clusters that contained the largest number of ESTs, and their distribution between parasitic and free-living stages, showed that 30 clusters accounted for 38% of the ESTs obtained and that 26 of these 30 clusters had significantly different representation between free-living and parasitic stages.

The ESTs obtained from the free-living stages have been used to construct a cDNA microarray with which we have interrogated gene expression in different larval stages of *S. ratti*. Significant differences in gene expression between different free-living larval stages were found, with the greatest difference (number of genes and magnitude of the fold difference in expression) between L1s and iL3s [18]. These analyses also showed that robust data could be generated that supported the computationally determined contig and cluster analysis of the ESTs. However, inconsistencies were found between EST representation data and microarray hybridisation data in the identification of genes with stage-specific expression and highly expressed genes. Further, rather little evidence for conservation of transcriptional profiles between *S. ratti* and *S. stercoralis* or *C. elegans* was found [18].

Here we report the construction of a cDNA microarray for the parasitic stages of *S. ratti* and its use to investigate changes in the gene expression of these stages that occur during an infection of a rat host. Significant changes in gene expression were observed, though the number of genes and the magnitude of the change in the level of expression were relatively small. We have combined these microarray data with our previous EST analysis, to identify *S. ratti* genes that are likely to be particularly important for the parasitic life of *S. ratti*, which we refer to as 'parasitism-central' genes. We suggest that these should be the first priority for further study in understanding the parasitic life of *S. ratti* and other nematodes.

2. Materials and methods

2.1. Parasite material

The *S. ratti* isofemale line ED321 Heterogonic was used and was maintained in female Wistar rats, as previously described [4,19]. Parasitic females were harvested from the intestine of sacrificed rats at 6 and 15 days post-infection (p.i.), and cleaned on a Percoll gradient, as previously described [9]. The parasitic females were concentrated in 200 μl media to which an equal volume of TRI reagent (Sigma Genosys Ltd., UK) was added, which was then snap frozen in liquid nitrogen and subsequently stored at −80 °C, until required [9].

2.2. Analysis of gene expression

We wished to determine the changes in gene expression that occurred in *S. ratti* parasitic females as an infection progressed, and thus as these stages are exposed to an anti-*S. ratti* immune response. To do this, we compared gene expression in parasitic females recovered 6 days p.i. (*i.e.* no or very low immune response) with those recovered at 15 days p.i. (*i.e.* high immune response); for convenience, we refer to these as parasitic females subject to 'low immune pressure' and 'high immune pressure', respectively. These days were chosen because previous analyses of *S. ratti* parasitic females have shown significant differences in the size, appearance, *etc*. of worms at these time points [9,20]. The experimental design used, was to have at least three biological replicates for each sample (*i.e.* three independent preparations of the relevant worm samples and their RNA) and to have at least three technical replicates (*i.e.* independent, separate cDNA synthesis, amplification and hybridisation, *etc.*) for each biological replicate. For each hybridisation (below) a dye-swap was used, *i.e.* each sample to be used in a hybridisation was labelled, separately, with each of the two dyes (below).

2.3. Microarray production

Twenty one thousand and eighty-five ESTs were sequenced from various *S. ratti* stage-specific libraries, of which 14,761 resulted in sequence data above a quality threshold that were then submitted to public databases [16]. Eleven thousand five hundred and fifty-one clones were derived from the *S. ratti* parasitic libraries of which 7385 produced sequence data (above a quality threshold); all of these 7385 clones were arrayed together with a random sample of 1619 of clones for which no sequence data were available. These 7385 ESTs are highly redundant since they represent 2963 contigs and 2125 clusters, both including 1220 singletons (*i.e.* clusters or contigs containing only one EST). Notwithstanding this redundancy, they were used in the microarray construction for two reasons: (i) this approach was less error-prone than attempting to select a unique clone set and (ii) this in-built redundancy provides many replicates of individual contigs and clusters, which can be exploited in quality-control