International Journal for Parasitology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Clearance of schistosome parasites by resistant genotypes at a single genomic region in *Biomphalaria glabrata* snails involves cellular components of the hemolymph

Euan R.O. Allan [a,*], Benjamin Gourbal [b], Camila B. Dores [c], Anais Portet [b], Christopher J. Bayne [a], Michael S. Blouin [a]

[a] Department of Integrative Biology, College of Science, Oregon State University, Corvallis, OR, USA
[b] Université Perpignan Via Domitia, Interactions Hôtes Pathogènes Environnements UMR 5244, CNRS, IFREMER, Univ. Montpellier, F-66860 Perpignan, France
[c] Department of Biomedical Sciences, Oregon State University, Corvallis, OR, USA

ARTICLE INFO

Article history:
Received 12 July 2017
Received in revised form 23 August 2017
Accepted 31 August 2017
Available online xxxx

Keywords:
Schistosomiasis
Biomphalaria glabrata
Histology
Hemocyte
Resistance
Hemolymph transfer
Plasma transfer

ABSTRACT

Schistosomiasis is one of the most detrimental neglected tropical diseases. Controlling the spread of this parasitic illness requires effective sanitation, access to chemotherapeutic drugs, and control over populations of the freshwater snails, such as *Biomphalaria glabrata*, that are essential intermediate hosts for schistosomes. Effectively controlling this disease, while minimising ecological implications of such control, will require an extensive understanding of the immunological interactions between schistosomes and their molluscan intermediate hosts. Here we histologically characterise the clearance of schistosome larvae by snails that exhibit allelic variation at a single genomic region, the Guadeloupe resistance complex. We show that snails with a resistant Guadeloupe resistance complex genotype clear schistosomes within the first 24–48 h, and that this resistance can be transferred to susceptible snails via whole hemolymph but not cell-free plasma. These findings imply that Guadeloupe resistance complex-coded proteins help to coordinate hemocyte-mediated immune responses to schistosome infections in Guadeloupean snails.

1. Introduction

Schistosomiasis is a devastating and neglected tropical illness that is responsible for hundreds of thousands of deaths each year, and afflicts over 250 million people in the developing world (WHO, 2012, 2016). This disease continues to persist despite mass chemotherapeutic drug administration by the World Health Organization (WHO), primarily because there are no effective vaccines targeting schistosomes, sanitation is lacking in some developing regions, and other mammalians can act as reservoir hosts and perpetuate the disease. Schistosomes require an intermediate snail host for their larval miracidial stage to develop, and regions that are unable to control these snails exhibit exacerbations in schistosomiasis cases (Sokolow et al., 2016). Presently, the only areas where schistosomes have been completely eliminated are regions where these intermediate snail hosts are extirpated, which makes them an essential target for disease control (Sokolow et al., 2016).

In the New World, miracidia from *Schistosoma mansoni* infect the aquatic snail *Biomphalaria glabrata*, which goes on to shed cercariae capable of causing human disease. This snail species has innate immune defenses that can both specifically and non-specifically target schistosomes for destruction (Coustau et al., 2015). The first line of defense against miracidial penetration is the integument of the snail. The integument provides a physical barrier of connective tissue and ciliated microvillus cells that protect from invading pathogens (Adlard, 2003). Miracidia must break through this barrier before they can infect any given individual. Successful miracidia, and the sporocysts that consequently develop, are then exposed to potentially degenerating humoral factors and attacked by motile hemocytes in the hemolymph and other tissues (Hanington et al., 2010; Loker, 2010). When schistosomes are recognised by hemocytes in resistant snails, they are encapsulated and exposed to anti-microbial effectors including reactive oxygen species which destroy the schistosome (Bender et al., 2005; Loker, 2010). Miracidia and sporocysts have numerous ways of evading destruction by humoral factors and hemocytes. Notable evasion tactics involve obscuring sporocyst detection post-integument penetration (Peterson et al., 2009), and the

* Corresponding author.
E-mail address: euanroallan@gmail.com (E.R.O. Allan).

https://doi.org/10.1016/j.ijpara.2017.08.008
0020-7519/

production of oxidative scavengers to protect them from hemocyte-mediated damage if they are detected (Mourao Mde et al., 2009; Mone et al., 2011). Exposed snails that are able to recognise and efficiently degrade invading schistosomes can escape infection, and subsequently avoid transmitting the disease to humans (Portela et al., 2013; Pinaud et al., 2016).

Resistance of Guadeloupean *B. glabrata* (BgGUA) to Guadeloupean *S. mansoni* (SmGUA) has been shown to be strongly correlated with allelic variation in the Guadeloupe resistance complex (GRC), but not with mRNA expression levels of these genes (Tennessen et al., 2015b). The GRC is a gene region which contains novel genes with vast amino acid sequence differences and putative immune recognition functions. *Grctm6* is a novel GRC-encoded protein that has been shown to affect the number of cercariae shed per infected snail and is expressed in hemocytes (Allan et al., 2017b). Understanding the kinetics and biology of this new class of genes in schistosome defense is essential before they might be used in alternative schistosome control. Although these studies have highlighted the importance of the GRC during schistosome infection, little is known about the actual mechanisms by which snails bearing resistant genotypes differ from those bearing susceptible genotypes. Determining when resistant genotypes clear parasitic larvae, and whether cellular or humoral factors are involved in clearance, is essential to understanding the mechanistic role of the genes in the GRC.

Histological differences between highly resistant and highly susceptible strains of snails have been characterised extensively, but these studies did not describe the genes that may cause the differences between those strains (Richards and Minchella, 1987; Borges et al., 1998; Loker, 2010; Nacif-Pimenta et al., 2012). Similarly, both cellular and humoral factors have been shown to transfer resistance from highly resistant to highly susceptible strains, but again no study has linked this transfer to allelic variation in specific genes (Sullivan et al., 1995, 2004; Vasquez and Sullivan, 2001a,b,c; Pinaud et al., 2016). In the present study, we report that snails bearing resistant GRC haplotypes have fewer unencapsulated sporocysts than snails bearing susceptible haplotypes as early as 24 h p.i., despite having equivalent integument structure and susceptibility to penetration by miracidia. We also show that resistance can be transferred from resistant GRC genotypes to susceptible GRC genotypes via injections of the resistant haplotype's hemolymph but not cell-free plasma. These findings demonstrate that GRC-mediated resistance to schistosome exposure occurs during the early stages of infection and is likely cell-mediated.

2. Materials and methods

2.1. Maintenance, propagation, infection and inbred line derivation of *B. glabrata* and *S. mansoni*

Biomphalaria glabrata (BgGUA) and *S. mansoni* (SmGUA) were collected in 2005 from Dans Fond (Theron et al., 2008) on the island of Guadeloupe, and maintained as previously described (Theron et al., 2014; Tennessen et al., 2015b). The SmGUA strain of *S. mansoni* was cycled through BgGUA and hamsters, and parasites were isolated from hamster livers or from shedding snails. BgGUA snails were segregated based on their genotype at the GRC locus, and separated into nine independently derived inbred lines as previously described: three *RR* (resistant lines R^1, R^2, R^3), three *S1S1* (susceptible lines $S1^1$, $S1^2$, $S1^3$), and three *S2S2* (susceptible lines $S2^1$, $S2^2$, $S2^3$) (Tennessen et al., 2015b; Allan et al., 2017b). To produce these lines, independent founder snails, homozygous at the GRC locus, were allowed to self-fertilise for three generations. For all additional experiments, equal numbers of snails pooled from each of the three lines within a GRC genotype were used (*RR*, *S1S1*, *S2S2* or *RR*, *SS*; where *SS* is pooled *S1S1* and *S2S2*). Experiments were performed on size/age matched (~7 mm, ~7 weeks old) cohorts of snails which were housed identically. This research adheres to Public Health Service Domestic Assurance for humane care and use of laboratory animals (PHS Animal Welfare Assurance Number A3229-01), as Animal Care and Use Proposal 4360, and was approved by the Oregon State University Institutional Animal Care and Use Committee, USA.

2.2. Confirmation of infection phenotypes in BgGUA lines

In order to verify that the inbred lines of BgGUA that were to be used for functional work behaved phenotypically like outbred snails of the same genotype, we examined the infection phenotype of all inbred lines using the same exposure protocol that was used on outbred BgGUA (Tennessen et al., 2015b). Parasite exposures of BgGUA with SmGUA were carried out as previously described (Tennessen et al., 2015b; Allan et al., 2017b). Snails were incubated in 2 ml of dechlorinated water in individual wells of a 24 well plate containing 20 miracidia for 24 h, and then transferred into tanks containing 10–15 snails each to be monitored for infection. Three independent lines each of *RR* (R^1, R^2, R^3), *S1S1* ($S1^1$, $S1^2$, $S1^3$), and *S2S2* ($S2^1$, $S2^2$, $S2^3$) snails were used to verify the susceptibility of these lines (Fig. 1). Infections were done on a minimum of two separate occasions with the total number of snails that were exposed exceeding 27 for any given line or treatment (n = 32 $S1^1$, 32 $S1^2$, 28 $S1^3$, 35 $S2^1$, 33 $S2^2$, 27 $S2^3$, 55 R^1, 31 R^2, 42 R^3). Exposed snails were scored for cercarial shedding by placing individual snails in 2 ml of dechlorinated water in a 24 well plate for 3 h, and scored as either infected or uninfected. This was done once per week between weeks 5–10 post exposure as previously described (Hanington et al., 2010, 2012; Pila et al., 2016b; Allan et al., 2017b).

2.3. Histological analysis

We histologically examined exposed snails of each genotype to determine the extent of early sporocyst development at different time points. Additionally, we examined the histological structure of the integument to ascertain if any difference in this outer barrier could explain differences in the infection phenotype of the GRC genotypes. For histological analysis of infected snails, BgGUA that were exposed to 20 miracidia were collected after 24 h, 48 h, or 10 days, and removed from their shells and fixed as previously described, with some modifications (Pinaud et al., 2016). In brief,

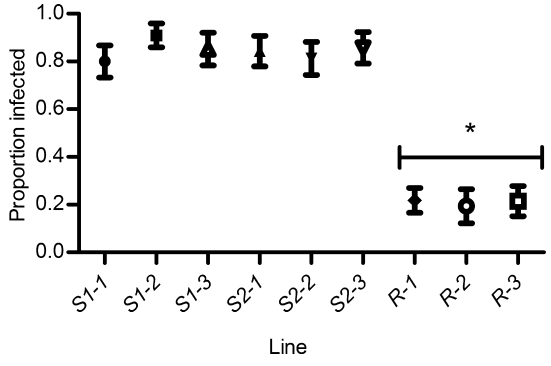

Fig. 1. Resistance phenotypes are conserved among inbred Guadeloupean *Biomphalaria glabrata* (BgGUA) lines. The susceptibility of nine independent homozygous BgGUA lines (3× *S1S1*, *S2S2* and *RR*) after exposure to 20 Guadeloupean *Schistosoma mansoni* (SmGUA) miracidia (n = 32 $S1^1$, 32 $S1^2$, 28 $S1^3$, 35 $S2^1$, 33 $S2^2$, 27 $S2^3$, 55 R^1, 31 R^2, 42 R^3). Data are presented as the proportion of infected snails (± the S.E. of proportions). Significant differences (P < 0.05, Z score of proportion) are denoted by asterisks (*).

Please cite this article in press as: Allan, E.R.O., et al. Clearance of schistosome parasites by resistant genotypes at a single genomic region in *Biomphalaria glabrata* snails involves cellular components of the hemolymph. Int. J. Parasitol. (2017), https://doi.org/10.1016/j.ijpara.2017.08.008