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RESEARCH ARTICLE

Life history adjustments to intestinal inflammation in a gut nematode

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ABSTRACT

Many parasitic nematodes establish chronic infections. This implies a finely tuned interaction with the host immune response in order to avoid infection clearance. Although a number of immune interference mechanisms have been described in nematodes, how parasites adapt to the immune environment provided by their hosts remains largely unexplored. Here, we used the gastrointestinal nematode *Heligmosomoides polygyrus* to investigate the plasticity of life history traits and immunomodulatory mechanisms in response to intestinal inflammation. We adopted an experimental model of induced colitis and exposed worms to intestinal inflammation at two different developmental stages (larvae and adults). We found that *H. polygyrus* responded to intestinal inflammation by up-regulating the expression of a candidate gene involved in the interference with the host immune response. Worms infecting mice with colitis also had better infectivity (earlier adult emergence in the intestinal lumen and higher survival) compared with worms infecting control hosts, suggesting that *H. polygyrus* adjusted its life history schedule in response to intestinal inflammation.

KEY WORDS: Adaptation, Infectivity, Inflammatory response, Phenotypic plasticity

INTRODUCTION

The interaction between hosts and parasites is based on an intimate molecular and physiological dialog between the two partners (Allen and Maizels, 2011). By definition, hosts and parasites have conflicting interests, with parasites exploiting the resources provided by the host and the host avoiding the cost of the infection. Avoiding the cost of infection can be achieved by several mechanisms. Hosts might behaviorally reduce the risk to contract the infection (Villa et al., 2016), might mount a vigorous immune response and clear the infection (Hansen et al., 2013), might tolerate the infection (Schneider and Ayres, 2008), or might adjust their life history traits (Agnew et al., 2000). Obviously, parasites have evolved counter-responses to each of these host defenses. In this respect, the capacity of parasites to interfere with the host immune response is one of the most prevalent exploitation strategies (Schmid-Hempel, 2008). This makes sense, as to a certain extent, the immune response can be seen as the main source of external mortality for the parasite and any strategy limiting the impact of the immune response on parasite survival should confer a selective advantage in terms of transmission potential.

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Helminth parasites, such as nematodes, have been described as having very sophisticated mechanisms of immune interference (Maizels and McSorley, 2016). The reasons that have probably promoted the evolution of such immune evasion strategies are multiple. Helminths are large metazoans, presenting many antigenic targets to the host immune system. Given their size, they also mechanically damage host tissues during their migration from the 'entrance' site to the targeted organ, which also contributes to the activation of the host immune response (Allen and Wynn, 2011). Helminth parasites usually establish chronic infections and their life history traits usually reflect a strategy of long-term exploitation with long pre-patent (the period preceding reproduction) and patent (the period during which the parasite reproduces within the host) periods (Morand and Sorci, 1998). Therefore, a successful parasite has to cope with the host immune response during relatively long periods of time, during different developmental stages and in different host tissues.

Heligmosomoides polygyrus is a rodent nematode that has become a model organism for the study of the molecular dialog between hosts and parasites (e.g. Urban et al., 1991; Shea-Donohue et al., 2001; Ince et al., 2009; Hang et al., 2010; Reynolds et al., 2012). Clearance of the infection depends on the capacity of the host to mount a Th2 immune response, with mouse strains failing to produce the appropriate response being highly susceptible to the infection (Reynolds et al., 2012).

The mechanisms underlying *H. polygyrus* adaptation to the host environment involve (i) its capacity to interfere with the host immune response (Maizels and McSorley, 2016); (ii) the microevolutionary response to immune selection; and (iii) the plastic adjustment of life history traits. Immune interference relies on parasite-induced upregulation of T-regulatory lymphocytes (Treg cells) which dampen both Th1 and Th2 immunity (Setiawan et al., 2007; Grainger et al., 2010). At the molecular level, it has been shown that *H. polygyrus* secretes and excretes a number of effectors that are involved in the process of immunomodulation (Maizels et al., 2012a; Valanparambil et al., 2014). More than 350 proteins have been characterized among the excreted products (Hewitson et al., 2011; McSorley et al., 2013), and several of them have candidate immunomodulatory properties (e.g. apyrase, chitinase, galactin, serpin). In this context, of particular interest are a homolog of the mouse anti-inflammatory cytokine transforming growth factor beta (TGF-β, coded by the *Hp-Tgh2* gene), and a cystatin (coded by the *Hp-CPI* gene) (Hewitson et al., 2009; McSorley et al., 2013). The homolog of the TGF-β has immunomodulatory functions through the suppression of T- and B-cell proliferation, the induction of Foxp3+ Tregs, the suppression of antigen-presenting cell maturation and TLR signaling (Grainger et al., 2010). Cystatin plays a role in the inhibition of antigen presentation and contributes to the suppression of pathology in experimental models of autoimmunity (Manoury et al., 2001; Schierack et al., 2003; Schnoeller et al., 2008; Sun et al., 2013).

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Parasite adaptation can also proceed through genetic selection. Pioneering work showed that *H. polygyrus* can adapt to the immune environment provided by the host (Su and Dobson, 1997). Serial passage experiments where *H. polygyurs* has been maintained in naïve or immunocompetent (previously infected) hosts for more than 30 generations has, for instance, shown that parasites passaged in previously infected mice had higher egg output, and better survival compared with the line maintained in naïve hosts (Su and Dobson, 1997).

Finally, parasite adaptation to the environment provided by their hosts can be achieved by plastic responses. Phenotypic plasticity usually evolves when the environment varies unpredictably (Fusco and Minelli, 2010). As heterogeneous host immune response is a pervasive phenomenon, helminths probably face variable immune selection within and between hosts, making such organisms well suited for the study of phenotypic plasticity (Viney and Diaz, 2012; Lippens et al., 2016). Plastic adjustments of *H. polygyrus* life history traits in response to the immune environment have recently been reported in a model of induced colitis. Donskow-Łysoniewska et al. (2013) treated mice with dextran sulphate sodium (DSS), which induces intestinal inflammation, and investigated the consequences of the altered cytokine milieu on *H. polygyrus* traits. They found that worms in an inflamed intestine had improved larval and early adult survival up to day 15 post-infection compared with worms in control hosts.

Although this last finding suggests that inflammatory conditions might prove beneficial for the parasite by making, for instance, worm expulsion harder, it raises a question. Indeed, *H. polygyrus* has also been reported, in several independent studies, to alleviate inflammatory symptoms (for instance in several models of induced colitis) (Elliott et al., 2004; Sutton et al., 2008; Blum et al., 2012; Donskow-Łysoniewska et al., 2012; Hang et al., 2013). Why then does *H. polygyrus* dampen the inflammatory response if an inflamed intestine possibly provides more favorable conditions for its establishment?

In this study, we wished to investigate this question and suggest that inflammatory conditions might be beneficial during the early phase of the infection for at least two different reasons. Early stages of the infection might benefit from an up-regulated Th1 pro-inflammatory status because (i) Th1 response tends to inhibit the Th2 response that is harmful for the parasite (Abbas et al., 1996) and (ii) it might help larvae to disrupt the intestinal wall and allow their penetration. In agreement with the hypothesis that immunomodulation confers a benefit to the parasite at later stages of the infection, recent work has shown that the production of the TGF-β homolog is stage specific, being minimal for pre-hatching and L1 larvae and progressively increasing to reach maximal production in adults (McSorley et al., 2010). Although McSorley et al. (2010) did not explicitly correct for worm biomass, they used different numbers of larvae and adults for their cDNA preparation (around 1000 larvae and 50 adults). Given the size difference between adult worms and larvae (between 10 and 20 mm for adults depending on the sex, and 300 and 600 µm for larvae), a ratio of 20:1 should somehow buffer the potential confounding effect of parasite growth on TGF-β homolog production. This result, therefore, suggests that the fitness consequences of immunomodulation might also be stage or age specific. In addition to this, if inflammation represents a 'risk' for the parasite, possibly reducing its life expectancy (as suggested by the age-dependent increase in the expression of the TGF-β homolog), selection should favor individuals that plastically adjust their life history schedule and increase their investment into traits expressed in early life, such as developmental time and early survival (Babayan et al., 2010).

To explore these questions, we used a rodent host model where colonic inflammation was induced by the ingestion of DSS. DSS is a water-soluble sulphated polysaccharide widely used as an inducer of colitis in mice. Its mechanisms of action involve the damage of the intestinal epithelium and the release of pro-inflammatory material into the underlying tissue (Chassaing et al., 2014). As a consequence, DSS administration alters the cytokine milieu, up-regulating pro-inflammatory mediators such as TNF-α, IL-6 and IL-17 (Alex et al., 2009). Moreover, DSS does not appear to be directly toxic for *H. polygyrus* (Donskow-Łysoniewska et al., 2013).

We designed an experimental set-up that allowed us to test the effect of intestinal inflammation on larvae (hosts were infected with *H. polygyrus* larvae when already undergoing the DSS treatment) and on freshly emerged adult worms (the DSS treatment started when adult nematodes emerged in the intestinal lumen). We thereafter assessed worm life history traits and the expression of two candidate genes involved in the immunomodulatory process (*Hp-tgh2* and *Hp-CPI*) in experimental and control groups. Based on the finding that the excretion of immunomodulatory products is stage specific in *H. polygyrus* (McSorley et al., 2010), we predicted that exposure to DSS should improve infectivity and worm survival during the early phase of the infection.

MATERIALS AND METHODS

Ethics statement

All animal experiments were approved by the Comité d'Ethique de l'Expérimentation Animale Grand Campus Dijon, France (CNREEA n C2EA – 105) (project N7801) in accordance with the national guidelines (Charte nationale portant sur l'éthique de l'expérimentation animale) on the use of animals for research purposes.

Animals

One hundred and forty female BALB/cJRj mice were purchased from Janvier Labs (Laval, France) and housed [five individuals per cage (18.5×38×22.5 cm); cages had shelters] at the Université de Bourgogne, France. Mice were maintained under a constant temperature (24°C) and a photoperiod of 12 h:12 h light:dark. They were fed with standard mouse pellets *ad libitum* and had *ad libitum* access to filtered tap water. Mice were weighed (±0.1 g) every 2 days.

At the age of 7 weeks, mice were infected by gavage with stage 3 larvae (120 larvae in 0.1 ml of water) of the intestinal nematode *H. polygyrus bakeri* (the parasite was kindly provided by Professor Rick Maizels, University of Edinburgh). L3 larvae were obtained from B6CBAF1 mice as the maintenance hosts. Upon ingestion, L3 larvae penetrate and encyst in the serosa of the duodenal wall where they undergo a couple of molts before emerging in the intestinal lumen at approximately day 8 post-infection.

Treatments

Mice (total N=140) were randomly allocated to one of five different experimental groups. Mice infected with *H. polygyrus* were treated with DSS (40 kDa) in drinking water (at 2.5%) over 8 days. In one group, mice (N=20) were exposed to DSS 3 days before *H. polygyrus* infection (in this group, larvae were exposed to DSS). In the other group, mice (N=30) were exposed to DSS at day 8 post-infection, when the first adults emerge in the intestinal lumen (in this group, freshly emerged worms faced the DSS). Another group of mice (N=40) was infected with the same number of *H. polygyrus* larvae but was not treated with DSS, and served as a first control

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group. The other two control groups were non-infected mice treated with DSS (8 days at 2.5%, N=20), and non-infected non-DSS treated mice (N=30).

Mice were killed by cervical dislocation, under isoflurane anaesthesia, at different time points to collect spleens and adult worms in the intestine. Spleens were immediately frozen in liquid nitrogen and then stored at −80°C. Adult worms were collected from the intestinal lumen and either immediately frozen in liquid nitrogen for gene expression analysis or stored in individual tubes for further counting.

Two mice in the group where worms underwent DSS treatment at the larval stage and five mice in the group where worms underwent DSS treatment at the adult stage showed rapid signs of health deterioration and were therefore killed before the expected culling time point.

Parasite life history traits

Adult worms were collected from the intestinal lumen and counted under a stereo microscope (×10). In the group where larvae were exposed to inflammation, we counted parasite numbers at days 9 and 12 post-infection (p.i.). In the group where adult worms were exposed to the inflammation, we counted parasite numbers at days 12, 16 and 20 p.i.

To assess fecal egg count, mice were transferred at 09:00 h on a grid in an individual cage with a humidified paper towel at the bottom to prevent feces desiccation. Mice were left for 4 h in these cages and then put back into their shared cages. Feces produced during this 4 h period were collected and 350 mg were smashed and suspended in 2.5 ml of water. Thereafter, 5 ml of salted water (75% saturation, 0.27 g NaCl per milliliter of water) was added to allow eggs to float. After agitation, a fraction of this suspension was transferred into a McMaster chamber for egg counting. We performed two counts per sample and used the mean values. Fecal egg count is expressed as number of eggs per milligram of feces. We also computed per capita fecundity as the ratio between the fecal egg count and the number of female worms recovered in the intestine.

Nucleic acid extraction and real-time PCR

Total mRNA was extracted from about twenty *H. polygyrus* adults per mouse and from the spleen (3 mm^3) using a TRIzol/chloroform protocol as recommended by the manufacturer (Invitrogen, Carlsbad, CA, USA). In both cases, RNA was precipitated using isopropanol, washed with 75% ethanol, and re-suspended in 50 µl of RNase-free water. We removed any potential gDNA contamination using DNAse treatment and removal reagents Ambion kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol, except for spleen samples because for each pair of primers, at least one overlapped two exons and was therefore RNA specific. RNA extractions were electrophoresed on 2% agarose gels and visualized using ethidium bromide staining. Degraded samples of RNA extractions were eliminated. Total RNA concentration was then estimated using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). We normalized concentrations to 250 ng μl^{-1} with RNase-free water and stored them at −80°C. A cDNA bank was generated from 2 µl of extracted RNA (500 ng per reaction) in a 20 µl reaction using an Improm-II reverse transcription system kit (Promega, Madison, WI, USA), following the manufacturer's protocol.

We quantified *Hp-Tgh2*, *Hp-CPI* and mouse-*TGF-β* expression by performing a real-time polymerase chain reaction (RT-PCR) using an Applied Biosystems StepOne Plus thermocycler (Applied Biosystems), in triplicate (triplicate PCRs from the same DNA sample) for each sample and gene. The PCRs were run on a total volume of 20 µl including 10 µl SYBR Green Master Mix 2X (Applied Biosystems), 0.5 µmol l^{-1} of each primer, 1 µl of cDNA diluted 20 times and RNAse/DNase-free water to complete the total volume. The PCR amplification was as follows: a first denaturation step at 95°C for 10 min, then 40 PCR cycles including denaturation and annealing step at 95°C for 15 s and elongation at 60°C for 1 min. To check the specificity of the assay, melting curves for all reactions were determined. This procedure consisted of incubations for 15 s at 95°C, 60 s at 60°C and a final slow heating with a rate of 0.3°C per second up to 95°C with continuous fluorescence measurement. A negative control that went through both the RNA extraction step and the cDNA bank construction was also added to each plate to ensure the absence of any contamination during the entire procedure. We used *Hp β-actin* as advocated by McSorley et al. (2010) and the mouse *β-actin* as housekeeping genes.

Hp primers were designed or retrieved from McSorley et al. (2010): *Hp β-actin*-F: 5′-TGA GCA CGG TAT CGT CAC CAA C; *Hp β-actin*-R: 5′-TTG AAG GTC TCG AAC ATG ATC TG (171 bp); *Hp-Tgh2*-F: 5′-CGG TGT GTC TGC CTG AAG AT; *Hp-Tgh2*-R: 5′-CGT TGT ATT TGT GCG GTG CA (108 bp); *Hp-CPI*-F: 5′-CTC GCC TCG TCT ATC GTC AC; *Hp-CPI*-R: 5′-CCG CCT TCC ATG CTT TTT CC (100 bp). Mouse primers were designed from the *Mus musculus domesticus β-actin* (NM_007393.5) and the *Tranforming Growth Factor-β1* (*TGF-β*) (NM_011577.2) RNA sequences, using the primer designing tools of the NCBI platform (www.ncbi.nlm.nih.gov/tools/primer-blast/); mouse-*β-actin*-F: CGA GCA CAG CTT CTT TGC AG; mouse-*β-actin*-R: CAT CCA TGG CGA ACT GGT G (65 bp); mouse-*TGF-β*-F: GAC CGC AAC AAC GCC ATC; mouse-*TGF-β*-R: GGG ACA GCA ATG GGG GTT C (112 bp).

Hp-Tgh2, *Hp-CPI* and *TGF-β* mRNA relative expression levels were estimated for each sample using the method developed by Pfaffl (2001). We calculated the relative expression (Qn) as:

$$Qn = \frac{[(E_{\text{tar}} + 1) - CP_{\text{tar}}]}{[(E_{\text{ref}} + 1) - CP_{\text{ref}}]}, \quad (1)$$

with E_{tar} and E_{ref} being the mean efficiencies of the target gene and the reference gene, respectively; and CP_{tar} and CP_{ref} being the crossing points of the target gene and the reference gene, respectively. The mean PCR efficiencies were estimated using LinRegPCR software (Ruijter et al., 2009). Crossing point values were estimated for each sample using the second derivative maximum method implemented in StepOne software (Thermo Fisher Scientific). We estimated, and considered in the analysis, mean *CP* corresponding to the average of triplicate RT-PCR reactions for target and reference genes.

Statistical analyses

The log-transformed *Hp-CPI*, *Hp-tgh2* and *TGF-β* gene expression relative to *β-actin* expression was analysed using an ANCOVA model with time post-infection, group and their interaction. The number of adult worms recovered in the intestinal lumen was analysed using an ANCOVA model with time post-infection, group and their interaction. The log-transformed parasite fecal egg count was analysed using a mixed model with mouse identity as a random factor (to account for the repeated nature of the data) and time post-infection, group and their interaction as fixed factors. Degrees of freedom were calculated using the Kenward–Roger method. For the group where adult worms were exposed to the DSS treatment, fecal

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egg count was monitored over six different days, which allowed inclusion of squared time as a fixed factor to model a non-linear effect. Per capita fecundity was analysed with an ANCOVA model with time post-infection, group and their interaction. Mouse body mass was analysed using a mixed model with mouse identity as a random factor and time post-infection, squared time post-infection, group, and their interactions as fixed factors. Degrees of freedom were calculated using the Kenward–Roger method.

For all analyses we started with the full model (with all possible interactions) and we subsequently dropped non-significant terms. We report the results of the full and the minimal adequate models. Differences in sample size reflect missing values due, for instance, to small amount of genetic material for RT-PCR, or individuals that were killed during the course of the study.

All analysis were performed with R software (version 3.0.3) including 'lme4' and 'car' packages, respectively, for mixed model and type 3 analysis of variance.

RESULTS

Genes whose products are putatively immunomodulatory

When worms were exposed to the DSS as larvae, we did not find any difference in the expression of *Hp-thg2* and *Hp-CPI* genes between groups (Table 1, Fig. 1A). We found that worms exposed to the DSS treatment as adults had a higher expression of the *Hp-thg2* gene (which codes for the *TGF-β* mimic) compared with the control environment. Although gene expression overall increased over time (model including only time p.i., $F_{1,16}$=18.17, P=0.0006), the rate of increase was higher for worms in DSS-treated mice, resulting in a statistically significant group×time interaction (Table 1). The expression of *Hp-CPI* (which codes for a cysteine protease inhibitor) did not vary between groups when adults were exposed to the DSS treatment (Table 1).

Parasite abundance

We found that parasites exposed to the DSS treatment emerged earlier than the control group, as shown by the higher number of adults at day 9 p.i. (Wilcoxon two-sample test, z=2.33, P=0.020, N=9). This difference disappeared at day 12 p.i. (Wilcoxon two-sample test, z=−0.42, P=0.676, N=10), resulting in a statistically significant interaction between group and time p.i. (time p.i., $F_{1,15}$=9.03, P=0.0089; group, $F_{1,15}$=27.49, P<0.0001; time p.i.×group, $F_{1,15}$=17.43, P=0.0008; Fig. 2A). We also found that the number of adult worms did not differ between the two DSS groups (larval versus adult exposure to DSS) at day 12 p.i. (Wilcoxon two-sample test, z=1.46, P=0.144, N=10).

The DSS treatment also had a strong effect on parasite numbers when adults were exposed to it. While the number of worms declined in control mice (Kruskal–Wallis test, χ^2=8.82, P=0.012, N=15), the number of worms did not vary with time in DSS-treated mice (Kruskal–Wallis test, χ^2=5.05, P=0.080, N=12) (Fig. 2B), resulting in a statistically significant interaction between group and time p.i. (time p.i., $F_{2,21}$=21.48, P<0.0001; group, $F_{1,21}$=2.31, P=0.1431; time p.i.×group, $F_{2,21}$=13.10, P=0.0002). This shows that the DSS treatment had a positive effect on worm survival up to day 20 p.i.

Parasite fecal egg count and per capita fecundity

Larval exposure to the DSS treatment consistently improved fecal egg count compared with the control group ($F_{1,23.40}$=13.23, P=0.0014); fecal egg count also increased with time p.i. ($F_{1,46.86}$=37.56, P<0.0001) with a parallel increase in the two groups (time p.i.×group, $F_{1,47.20}$=0.17, P=0.6835; Fig. 3A).

When adult worms were treated with DSS, we also found an improvement in fecal egg count, even though the difference between the DSS and control group appeared at day 16 p.i. ($F_{1,14}$=21.99, P=0.0003). Whereas fecal egg count of the control group showed bell-shaped dynamics, fecal egg count of worms in the DSS-treated group remained high up to day 20 p.i. This resulted in a statistically significant interaction between group and time p.i. (Table 2, Fig. 3B).

Larval exposure to the DSS treatment did not improve per capita fecundity compared with the control group (time p.i., $F_{1,15}$=39.91, P<0.0001; group, $F_{1,15}$=0.94, P=0.3468; time p.i.×group, $F_{1,14}$=0.26, P=0.6196), suggesting that the effect on fecal egg count was essentially due to variation in population size. Similarly, when adult worms were exposed to the DSS treatment, their per capita fecundity did not differ between groups or over time (time p.i., $F_{1,22}$=0.27, P=0.6054; group, $F_{1,22}$=0.00, P=0.9478; time p.i.×group, $F_{1,21}$=1.14, P=0.2984).

Inflammation severity

DSS-induced colitis produces a series of symptoms on hosts and we used changes in body mass as an integrative index of inflammation severity. To this purpose, we had an additional group with mice that were only exposed to DSS but not infected by *H. polygyrus*, and a group of control mice (not exposed to DSS and not infected). Although control and *H. polygyrus*-infected mice gained body mass over the course of the study, exposure to DSS induced a loss in body mass (Table 3, Fig. 4). To investigate this result further, we ran models where the groups were compared two-by-two. These additional models showed that for all the comparisons between one of the inflammatory groups (DSS, DSS-larval exposure, DSS-adult exposure) and one of the control groups (control, *H. polygyrus*), the squared time×group interaction was always significant (all P<0.05); for all the comparisons between DSS, DSS-larval exposure and DSS-adult exposure, neither the squared time×group interaction, nor the time×group interaction were statistically significant (all P>0.12). This shows that the dynamics of body mass loss in the three inflammatory groups was very similar, suggesting that the infection had very little effect on colitis severity, at least based on changes in body mass.

Table 1. ANCOVA model of the effect of time post-infection, group and their interaction on the expression of *Hp-CPI* and *Hp-thg2* genes relative to *β-actin* in *Heligmosomoides polygyrus* exposed to dextran sulphate sodium (DSS) either as larvae or as adults

		Hp-CPI			*Hp-thg2*		
Parasite stage exposed to DSS	Source of variation	*F*	d.f.	*P*	*F*	d.f.	*P*
Larvae	Time p.i.	2.3215	1,10	0.1586	36.8348	1,10	0.0001
	Group	0.0613	1,10	0.8095	1.6243	1,10	0.2313
	Time p.i.×group	0.1836	1,10	0.6774	2.3089	1,10	0.1596
Adults	Time p.i.	0.0396	1,16	0.8464	1.0873	1,16	0.3126
	Group	2.1711	1,16	0.16	4.9057	1,16	0.0416
	Time p.i.×group	2.3807	1,16	0.1424	8.5277	1,16	0.0100

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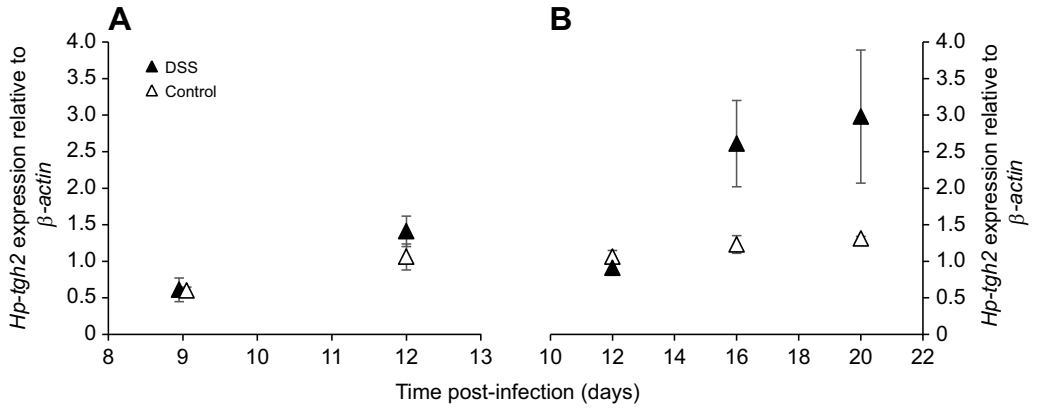

Fig. 1. *Hp-tgh2* expression relative to *β-actin* as a function of time post-infection in *Heligmosomoides polygyrus*. (A) When *H. polygyrus* larvae were exposed to the dextran sulphate sodium (DSS) treatment (filled triangles) or a control environment (open triangles), only time p.i. affected *Hp-tgh2* expression (P=0.0001, N=14); (B) when *H. polygyrus* adults were exposed to the DSS treatment (filled triangles) or a control environment (open triangles), *Hp-tgh2* expression was affected by the interaction between time p.i. and group (P=0.0100, N=20). Means±s.e.m. are reported.

Mouse *TGF-β* gene expression

We finally assessed the expression of *TGF-β* in the spleen of mice in the different groups. Mice in the different experimental groups had different *TGF-β* expression, whatever the timing of *H. polygyrus* exposure to the DSS treatment (larvae: time p.i., $F_{1,32}$=4.17, P=0.0496; group, $F_{3,32}$=3.69, P=0.0219; time p.i.×group, $F_{3,29}$=0.47, P=0.7042; adults: time p.i., $F_{1,49}$=1.05, P=0.3103; group, $F_{3,49}$=4.75, P=0.0055; time p.i.×group, $F_{3,46}$=0.19, P=0.9007; Fig. 5). Local tests showed that, at day 16 p.i., DSS-treated mice had a higher *TGF-β* expression compared with DSS-treated and *H. polygyrus*-infected mice (Tukey's studentized range test, P=0.039), suggesting that *TGF-β* in DSS-treated mice was down-regulated when they were concomitantly infected with *H. polygyrus*.

DISCUSSION

We found that intestinal inflammation affected the life history traits of the gut nematode *H. polygyrus*, and, overall, nematodes that faced an inflammatory response had better infectivity and survival during the early phase of the infection, compared with the control group.

We explored the adaptation of *H. polygyrus* to intestinal inflammation using a well-established colitis model induced by the administration of DSS. DSS alters the intestinal cytokine environment, upregulating the production of inflammatory cytokines and markers (Chassaing et al., 2014). Here, we assessed the expression of the *TGF-β* gene in host spleen and found that DSS administration enhanced its expression. This finding, in addition to the observed decrease in body mass in DSS-exposed mice, shows that we successfully altered the intestinal environment worms were exposed to. Interestingly however, the upregulation of the *TGF-β* gene involved the DSS-only group, whereas DSS-treated mice that were also infected with *H. polygyrus* had similar *TGF-β* expression to the control group. It is tempting to speculate on a possible co-evolutionary scenario here where mice concomitantly exposed to DSS and infected with *H. polygyrus* rely on the anti-inflammatory response provided by the parasite, whereas in the absence of the infection, mice can only rely on their endogenous anti-inflammatory response. In agreement with this view, *H. polygyrus* upregulated the expression of the gene coding for the *TGF-β* homolog (*Hp-tgh2*) when exposed to DSS. Further work should elucidate the link between endogenous and exogenous anti-inflammatory responses when facing an inflammatory insult. We also found that the expression of *Hp-tgh2* increased over worm age in control mice, in agreement with previous work (McSorley et al., 2010). However, the rate of *Hp-tgh2* expression increase over time was much higher for worms in the DSS group. This indicates an environmental-dependent induction of the *TGF-β* homolog and raises the question of the possible costs that might prevent an elevated constitutive (environmental-independent) gene expression. Although, we do not have direct evidence indicating possible costs of *Hp-tgh2* gene expression, in a companion work we showed that while the gene is readily up-regulated a few hours after mice have been challenged

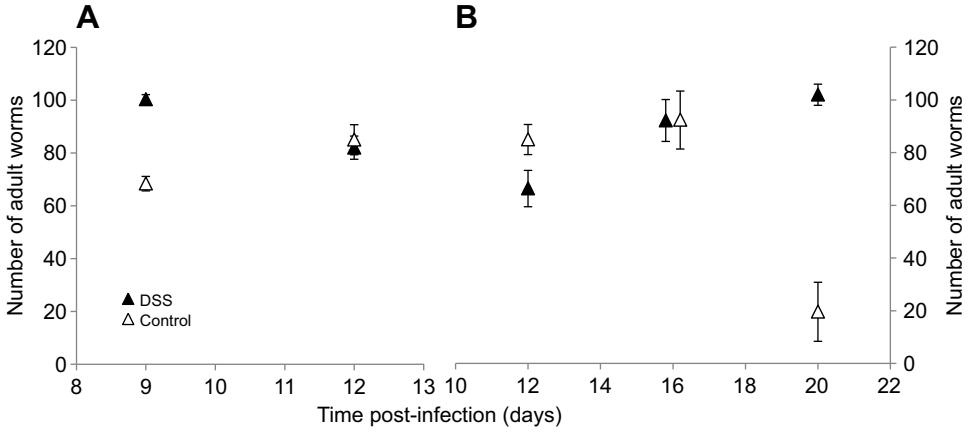

Fig. 2. Number of adult worms in the intestinal lumen as a function of time post-infection in *H. polygyrus*. (A) When *H. polygyrus* larvae were exposed to the DSS treatment (filled triangles) or a control environment (open triangles), the number of adult worms was affected by the interaction between time p.i. and group (P=0.0008, N=19); (B) when *H. polygyrus* adults were exposed to the DSS treatment (filled triangles) or a control environment (open triangles), the number of adult worms was affected by the interaction between time p.i. and group (P=0.0002, N=27). Means± s.e.m. are reported.

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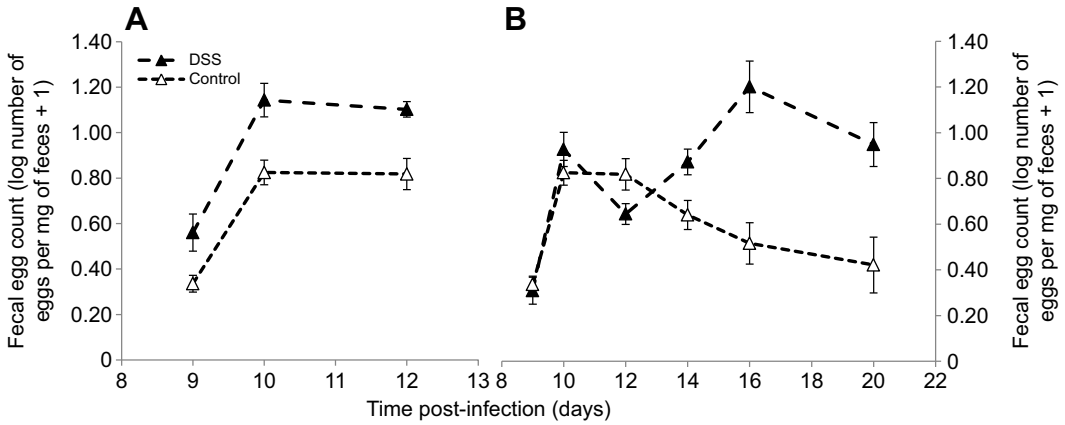

Fig. 3. Fecal egg count as a function of time post-infection in *H. polygyrus*. (A) When *H. polygyrus* larvae were exposed to the DSS treatment (filled triangles) or a control environment (open triangles), fecal egg count was affected by time p.i. (P<0.0001) and group (P=0.0014) (N=28); (B) when *H. polygyrus* adults were exposed to the DSS treatment (filled triangles) or a control environment (open triangles), fecal egg count was affected by the interaction between time p.i. and group (P=0.0041, N=35). Means±s.e.m. are reported.

with a lipopolysaccharide (LPS) injection, selection of worms in hosts treated with (i) a recombinant TGF-β, (ii) an anti-TGF-β antibody, or (iii) left as control does not affect the constitutive expression of *Hp-tgh2* when the different selected lines of worms are used to infect control hosts (Guivier et al., 2017). This result suggests that constraints prevent the evolution of a constitutively up-regulated expression of the *Hp-tgh2* gene.

Based on the finding that expression of the *Hp-tgh2* gene is age dependent in *H. polygyrus* (McSorley et al., 2010), we predicted that intestinal inflammation might enhance parasite performance during the early phase of the infection. This prediction was also rooted in a previous work that used a DSS model of induced colitis, showing that mice with inflamed intestine harbored more larvae and more adults compared with control mice (Donskow-Łysoniewska et al., 2013). In agreement with our prediction, we found that worms exposed to DSS emerged earlier and had better survival up to day 20 p.i. This suggests that exposure to DSS improved infectivity. Declining number of adult worms is a typical finding for BALB/c mice, owing to the progressive expulsion of worms under the joint action of the immune response and peristaltic movements (Zhao et al., 2008). Our finding that, in an inflamed intestine, worm survival was preserved suggests that the immune system of colitis mice failed to deal with the infection. The mechanisms underlying such a finding might involve a skewed immune response towards a Th1, away from the protective Th2 response (Maizels et al., 2012b). Alternatively, previous work has shown that exposure to intestinal inflammation can change the proteome profile of adult *H. polygyrus*, reducing immune recognition of parasites and improving survival (Donskow-Łysoniewska et al., 2013). As a consequence of higher parasite numbers, DSS-treated mice shed significantly more eggs than control hosts. However, per capita fecundity did not differ between groups, further showing that the effect of DSS was restricted to parasite infectivity.

Theory on the evolution of life history traits has suggested that living in a 'risky' environment, where individual longevity is potentially impaired, should select for higher investment into life history traits that are expressed in early life, promoting faster development and earlier age at reproduction (Stearns, 1992). It is therefore possible that worms facing intestinal inflammation adopted a faster pace of life, with accelerated development, enhanced early survival but increased late mortality. Such shifts in life history strategies have been documented in several species in response to threatening environmental conditions (Agnew et al., 2000). For instance, recent work has provided very convincing evidence for life history shifts in filarial nematodes infecting either immunocompetent mouse hosts or hosts lacking the appropriate immune response (Babayan et al., 2010). In the presence of IL-5-driven eosinophilia, which reduces parasite life expectancy, filarial worms have accelerated development and reproduce earlier (Babayan et al., 2010). Our results also fit into this general scenario, as DSS-exposed worms emerged earlier into the intestinal lumen (a proxy of age at first reproduction) and had higher survival

Table 2. Mixed model of the effect of time post-infection, squared time, group and the two-way interactions on fecal egg count (log-transformed) in *H. polygyrus* exposed to the DSS treatment as adults

Source of variation	F	d.f.	P
Full model			
Time p.i.	14.8669	1,111.33	0.0002
Squared time p.i.	15.0579	1,110.68	0.0002
Group	0.0017	1,109.89	0.9676
Time p.i.×group	0.0278	1,109.97	0.8679
Squared time p.i.×group	0.3164	1,110.85	0.5749
Minimal adequate model			
Time p.i.	19.8578	1,112.39	<0.001
Squared time p.i.	20.3032	1,111.58	<0.001
Group	5.2721	1,128.94	0.0233
Time p.i.×group	8.5533	1,123.23	0.0041

Table 3. Mixed model of the effect of time post-DSS exposure, squared time, group and the two-way interactions on host body mass when *H. polygyrus* was exposed to the DSS treatment as larvae or as adults

Parasite stage exposed to DSS	Source of variation	F	d.f.	P
Larvae	Time post-DSS	8.57	1,449.30	0.0036
	Squared time post-DSS	2.01	1,449.42	0.1568
	Group	0.47	3,123.88	0.7013
	Time post-DSS×group	11.77	3,448.46	<0.001
	Squared time post-DSS×group	5.78	3,448.76	<0.001
Adults	Time post-DSS	6.14	1,513.46	0.0135
	Squared time post-DSS	0.28	1,512.62	0.5946
	Group	2.73	3,170.98	0.0453
	Time post-DSS×group	3.82	3,510.85	0.0101
	Squared time post-DSS×group	13.97	3,511.29	<0.001

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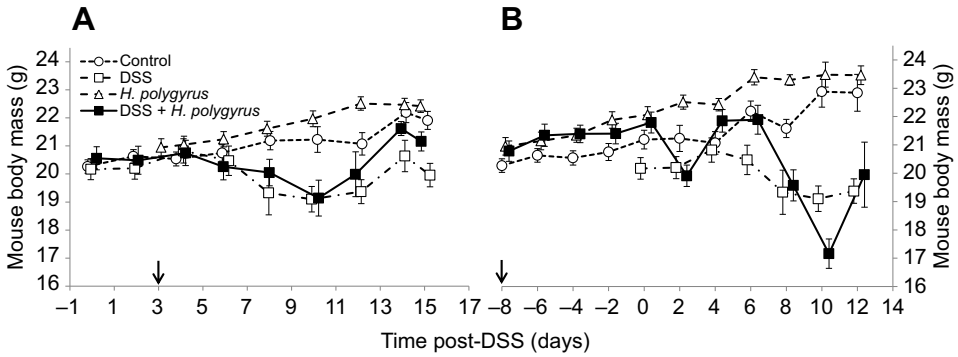

Fig. 4. Mouse body mass as a function of time post-DSS exposure for the different experimental groups. The two panels refer to the groups where *H. polygyrus* was exposed to the DSS treatment as larvae (A) or as adults (B). Open circles refer to the control group (initial *N*=30), open squares to the DSS-treated group (initial *N*=20), open triangles to the *H. polygyrus*-infected group (initial *N*=20) and filled squares to the DSS-treated and *H. polygyrus*-infected group (initial *N*=10 and 15). The arrow indicates the day when mice were infected with *H. polygyrus*. Whatever the timing of *H. polygyrus* exposure to the DSS treatment, mouse body mass was affected by the interaction between squared time post-DSS and group (both *P* values<0.0001). Means±s.e.m. are reported.

up to day 20 p.i. The underlying assumption is that these different life history trajectories finally produce similar fitness across different environments. To test this hypothesis, we would need to monitor parasite life history traits over the entire lifespan of the worms to confirm that higher investment into early expressed traits is traded against lately expressed traits (i.e. longevity). Although we did not monitor worm lifespan and lifetime egg output, support for the hypothesis that inflammation reduces worm longevity comes from other experiments where *H. polygyrus* was used to infect LPS-treated mice. Whereas LPS injection induced an increase in egg output in the few hours following the inflammatory challenge compared with the control group (Guivier et al., 2017), at day 90 p.i. LPS-treated mice shed a significantly lower amount of parasite eggs, and at day 210 p.i. 56% of LPS-treated mice had cleared the infection compared with 13% of control mice (Guivier et al., 2016). An additional possibility is that costs of facing inflammation are paid not only during the late phase of the infection but also during the following generation, as also suggested by Babayan et al. (2010). Intergenerational effects on *H. polygyrus* infectivity have recently been shown (Lippens et al., 2017a), making this hypothesis plausible.

The finding that exposure to DSS improves parasite infectivity raises several questions. Obviously the first one is related to the capacity of *H. polygyrus* to dampen the host immune response (Maizels et al., 2012a) and to alleviate the symptoms of intestinal inflammation (Hang et al., 2013). If an inflamed intestine provides a more suitable environment for the nematode, why does *H. polygyrus* dampen it? This key question remains open at the moment, and we can only speculate on possible explanations. As suggested here, intestinal inflammation might have different effects across different parasite life stages/ages, having positive effects during the early phase of the infection (the establishment phase) and negative ones during the chronic phase. The report that the production of the *TGF-β* homolog increases (i) from larvae to adults (McSorley et al., 2010) and (ii) over parasite age (this study) seems to support this view. Further lines of evidence also support the view that inflammation might incur costs during the late, chronic phase of the infection (Guivier et al., 2016). In addition to the direct positive effect of inflammation (inhibition of the Th2 response), life history shifts towards early expressed traits might, however, also reflect plastic adjustments to buffer reduced life expectancy. As *H. polygyrus* establishes long-lasting infections that can persist for months in permissive hosts, worm longevity is probably a key life history trait determining individual lifetime reproductive success.

Parasite adaptation to the immune environment provided by the host also raises the question of the evolutionary consequences in terms of the capacity of nematodes to successfully treat immune diseases and/or alleviate inflammatory symptoms. Previous reports

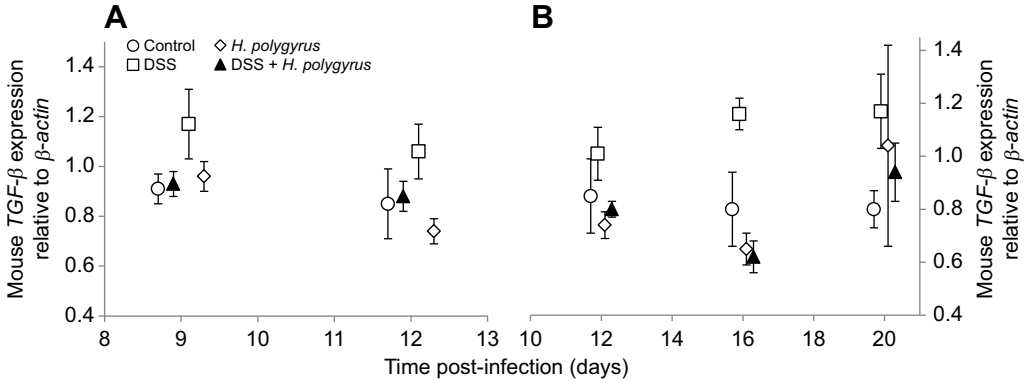

Fig. 5. Mouse *TGF-β* expression relative to *β-actin* as a function of time post-infection for the different experimental groups. The two panels refer to the groups where *H. polygyrus* was exposed to the DSS treatment as larvae (A) or as adults (B). Open circles refer to the control group (*N*=9 and 14), open squares to the DSS-treated group (*N*=10 and 14), open diamonds to the *H. polygyrus*-infected group (*N*=9 and 12), and filled triangles to the DSS-treated and *H. polygyrus*-infected group (*N*=9 and 14). Whatever the timing of *H. polygyrus* exposure to the DSS treatment, mouse *TGF-β* expression was affected by the group (*P*=0.0219 for larvae and *P*=0.0055 for adults). Means±s.e.m. are reported.

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that *H. polygyrus* can evolve according to the immune status of its host after a few generations of selection (Dobson and Owen, 1977) opens the fascinating possibility of engineering parasites with improved therapeutic properties (Lippens et al., 2017b).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: C.L., E.G., B.F., G.S.; Methodology: C.L., E.G., A.O.; Formal analysis: C.L.; Data curation: C.L.; Writing - original draft: G.S.; Writing - review & editing: C.L., E.G., B.F., G.S.; Funding acquisition: B.F., G.S.

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Data availability
Data are available from the Dryad Digital Repository (Lippens et al., 2017c): doi:10.5061/dryad.7mp24

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