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Plastic and micro-evolutionary responses of a nematode to the host immune environment

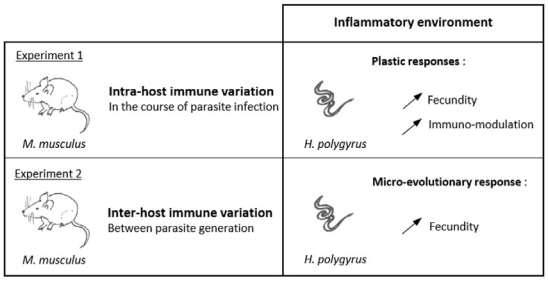
Emmanuel Guivier [a, b, *], Cédric Lippens [a], Bruno Faivre [a], Gabriele Sorci [a]

[a] Biogéosciences, CNRS UMR 6282, Université de Bourgogne Franche-Comté, 6 Bd Gabriel, 21000 Dijon, France
[b] Physiopathologie des dyslipidémies, INSERM UMR 866, Université de Bourgogne Franche-Comté, 21000 Dijon, France

HIGHLIGHTS

- Nematodes responses to Intra- and inter-host immune variability were investigated.
- *H. polygyrus* plastically responded to a sudden rise of pro-inflammatory cytokines.
- Parasites can adjust the expression of immunomodulatory genes to host immunity.
- Response to inflammation is also accompanied by an enhanced parasite egg shedding.
- Our results suggest selection on *Hp* life history traits in inflammatory environment.

GRAPHICAL ABSTRACT



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ABSTRACT

Parasitic organisms have to cope with the defences deployed by their hosts and this can be achieved adopting immune evasion strategies or optimal life history traits according to the prevailing pattern of immune-mediated mortality. Parasites often encounter variable immune environments both within and between hosts, promoting the evolution of plastic strategies instead of fixed responses. Here, we explored the plasticity and micro-evolutionary responses of immunomodulatory mechanisms and life history traits to the immune environment provided by the host, using the parasitic nematode *Heligmosomoides polygyrus*. To test if the parasite responds plastically to the immune environment, we stimulated the systemic inflammatory response of mice and we assessed i) the expression of two genes with candidate immunomodulatory functions (*Hp-Tgh2* and *Hp-CPI*); ii) changes in the number of eggs shed in the faeces. To test if the immune environment induces a micro-evolutionary response in the parasite, we maintained the nematode in mice whose inflammatory response was up- or down-regulated during four generations. We found that *H. polygyrus* plastically responded to a sudden rise of pro-inflammatory cytokines, up-regulating the expression of two candidate genes involved in the process of immune modulation, and enhancing egg output. At the micro-evolutionary level, parasites maintained in hosts experiencing different levels of inflammation did not have differential expression of *Hp-Tgh2* and *Hp-CPI* genes when infecting unmanipulated, control, mice. However, parasites maintained in mice with an up-regulated inflammation shed more eggs compared to the control line. Overall, our study shows

* Corresponding author. Current address: IMBE, Université Aix-Marseille, Centre St Charles, 13331 Marseille Cedex 3, France.
E-mail address: em.guivier@gmail.com (E. Guivier).

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that *H. polygyrus* can plastically adjust the expression of immunomodulatory genes and life history traits, and responds to selection exerted by the host immune system.

1. Introduction

In response to selection pressures exerted by the immune system, parasites have evolved fascinating strategies to evade immunity through physiological and biochemical mechanisms (Schmid-Hempel, 2008). Nematodes are among the best examples of parasites that can down-regulate the host immune response for their own benefit (Maizels et al., 2004).

Along the course of a nematode infection, the immune environment provided by the host is likely to change. The vertebrate immune response is a complex process with successive and interacting components (innate and acquired immunity), involving different cellular and molecular effectors (Akira et al., 2006). After its penetration into the host, a nematode faces the innate immune response (De Veer et al., 2007). Then, the recognition of parasite epitopes stimulates the Th2 immune pathway and the activation of the immune regulatory network (Gause, 2003). Thus, from its penetration into the host to its death, a parasitic nematode deals with different immune components which modify its immediate environment. Since many nematode species establish long lasting infections, they are also likely to encounter variable immune environments as long as the host cyclically engages in stressful activities (i.e., reproduction, migration) or has to deal with secondary pathogen infections. Parasitic nematodes have also to cope with spatial variation in their environment during their "migration" through different host tissues and organs that provide different immune environments (Read and Sharping, 1995). At the intra-host level, therefore, nematodes have to face spatial and temporal variation in immune environments.

Between generations, nematodes also deal with different immune environments. Host populations are characterized by a strong variation in the ability of individuals to mount an immune response (Hayward, 2013). This variability, shaped by genetic and ecological factors, promotes the coexistence of susceptible and resistant hosts to specific parasites. It also implies that from one generation to the other, parasites are likely to experience different immune environments, depending on whether they will end up infecting a resistant or a susceptible host. Thus, the benefits and costs of immunomodulatory strategies deployed by the parasite are likely to vary across generations.

In addition to directly interfering with the host immune response, parasites that establish long lasting infections might respond to perceived risk of immune-mediated mortality by adjusting the age-specific investment into reproduction. This is very similar to the predictions that models on the evolution of life history traits have provided for free-living organisms (Michod, 1979). Increased risk of mortality should produce a rise in current reproduction because saving energy for future reproduction might be worthless (Paterson and Barber, 2007).

Adaptation to specific environmental conditions is usually achieved through two main mechanisms: phenotypic plasticity and genetic selection. Theoretical work has shown that living in a variable and unpredictable environment should promote the evolution of phenotypic plasticity (Scheiner, 1993). Based on this theoretical prediction and the variability parasites have to face both within and between hosts, one can predict that gut nematodes i) should have evolved mechanisms allowing them to rapidly shift between different environmental types, through plastic responses (Viney and Diaz, 2012), both in terms of immunomodulatory strategies and life history trajectories (Lippens et al., 2016); ii) should adapt their investment into immune evasion effectors according to the experienced level of immune aggression.

Here, we tested whether parasitic nematodes exposed to a systemic inflammatory response have plastic immunomodulatory mechanisms and life history traits, and whether there is a micro-evolutionary response after four generations of selection under such specific immune environments. Although the induced activation of systemic inflammation does not represent a direct response to the parasite, it mimics the potential leakage of bacterial material during the nematode penetration into the intestinal wall, or any co-infection with opportunistic bacteria. To this purpose, we used the association between the gut nematode *Heligmosomoides polygyrus* and its rodent host, *Mus musculus musculus*. *Heligmosomoides polygyrus* releases a large number of excretory/secretory molecular products (Valanparambil et al., 2014). Proteomic and glycomic characterization has shown that more than 350 proteins are present in these ES products (Hewitson et al., 2011; McSorley et al., 2013). Although not all these proteins are involved in the process of immunomodulation, several are candidate immunomodulators. Among ES family products that have been shown to have immunomodulatory properties (including apyrase, chitinase, galactin, serpin), we focused on the expression of two genes: *Hp-Tgh2*, a homolog of the anti-inflammatory cytokine transforming growth factor beta (TGF-β), and *Hp-CPI*, a cystatin (Hewitson et al., 2009; McSorley et al., 2013). The immunomodulatory role of the TGF-β mimic involves the suppression of T and B cell proliferation, the induction of Foxp3+ Tregs, the suppression of antigen-presenting cell maturation and TLR signalling (Grainger et al., 2010). Cystatin inhibits antigen processing and presentation and it has been shown to suppress pathology in autoimmunity models (Manoury et al., 2001; Schierack et al., 2003; Schnoeller et al., 2008; Sun et al., 2013).

At the intra-host level, we experimentally modified the immune environment experienced by *H. polygyrus* by activating the inflammatory response of mice with the injection of *Escherichia coli* lipopolysaccharide (LPS). At the inter-host level (i.e. between generations of nematodes), we maintained *H. polygyrus* in hosts whose inflammation was up-regulated (mice treated with anti-TGF-β antibodies), down-regulated (mice treated with TGF-β) or left as unmanipulated control. TGF-β is one of the main suppressive cytokines with effects on lymphocyte proliferation and differentiation (Li et al., 2006). Previous work has shown that TGF-β and anti-TGF-β antibody injections in mice effectively down- and up-regulate immune effectors in response to infection (Beiting et al., 2007; Doligalska et al., 2006; Herbert et al., 2008; Li et al., 1999; Omer and Riley, 1998).

At the intra-host level, we predicted that the LPS challenge should produce a plastic response in terms of up-regulation of genes involved in immunomodulation. In terms of adjustment of egg shedding, two scenarios can be envisioned, each of them providing contrasting predictions. The first scenario is based on the idea that immune effectors limit the expression of parasite life history traits. This mechanistic view therefore posits that parasites in hosts with an up-regulated immune response should pay an