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Microevolutionary response of a gut nematode to intestinal inflammation

Cédric Lippens, Bruno Faivre, Gabriele Sorci *

Biogéosciences, CNRS UMR 6282, Université de Bourgogne Franche-Comté, France

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ABSTRACT

Parasitic helminths interfere with the immune responses of their hosts to establish long-lasting, chronic infections. While favorable to the parasite, the capacity to dampen the immune response can also provide a benefit to the host in terms of reduced risk of immune disorders and immunopathology. The immunomodulatory role of nematodes has been exploited in clinical trials to treat a number of inflammatory and immune diseases. However, how parasites adapt to an inflammatory environment remains a poorly explored question. Here, we conducted a serial passage experiment where the gut nematode *Heligmosomoides polygyrus* was maintained for nine generations in mice with a drug-induced intestinal inflammation or in control hosts. The life history traits of parasites from the selected lines were assessed in hosts that were either exposed to the inflammatory environment or kept as controls. In addition to the nematode life history traits, we assessed the severity of the intestinal inflammation. We found that *H. polygyrus* adapted to the inflammatory environment through both plastic and microevolutionary responses. In particular, per capita fecundity was globally enhanced in worms that experienced intestinal inflammation and that were selected in the inflammatory environment. Interestingly, we also found that worms selected in the inflammatory environment were better able, after nine generations of selection, to alleviate the inflammatory symptoms. This latter result further highlights the potential therapeutic role of gut nematodes in the treatment of inflammatory diseases.

1. Introduction

A common feature shared by virtually all parasitic organisms is that they have to cope with the defenses mounted by their hosts. Vertebrates have a complex and sophisticated anti-pathogen defense, the immune system. Parasites have evolved an astonishing diversity of mechanisms for immune evasion that range from hiding or becoming invisible to the immune system, to directly suppressing the immune response (Schmid-Hempel, 2008). Helminths are often put forward as being one of the best examples of parasites with immunosuppressive effects (Maizels et al., 2004). Compared with microparasites, helminths are large, complex, metazoans that offer many antigenic sites to immune cells. Their size often induces traumatic lesions during their penetration and migration within the host body, activating and stimulating the host immune response (Allen and Wynn, 2011). Finally, they have relatively long life cycles and can persist for years within their definitive hosts (Gems, 2000), which implies a long-lasting interaction with the host immune system. For all these reasons, the persistence of helminths in their definitive hosts requires a finely-tuned regulation of the immune response (Maizels and McSorley, 2016).

Helminthiases are still widespread infections of humans in tropical countries, while in Europe and North America infection with intestinal worms has almost disappeared (World Health Organization, 2009; Lustigman et al., 2012). Interestingly, the temporal trend of decline in the prevalence of helminthiasis in wealthy countries has been paralleled by a sudden increase in the incidence of immune and inflammatory diseases such as inflammatory bowel disease, multiple sclerosis and allergies (Bach, 2002). It is tempting, therefore, to speculate on the possible role that helminths have played as regulators of the human immune response during our evolutionary history and that their eradication has disrupted this fine-tuned equilibrium (Sorci et al., 2016). Even though most evidence is based on epidemiological associations between the incidence of immune diseases and the prevalence of infection

* Corresponding author at: Biogéosciences, CNRS UMR 6282, Université de Bourgogne Franche-Comté, 6 Boulevard Gabriel, 21000 Dijon, France.
E-mail address: gabriele.sorci@u-bourgogne.fr (G. Sorci).

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(Fleming and Cook, 2006), some experimental work supports the view that helminths are key in preventing the risk of immune disorders (see review by Finlay et al., 2014). At the molecular level, the mechanisms underlying the protective effects have been fairly well established (Broadhurst et al., 2010, 2012; McSorley et al., 2013; Finlay et al., 2014; Heylen et al., 2014; Bashi et al., 2015). Obviously, the capacity of helminths to protect from, and alleviate, the symptoms of immune diseases paves the way for a possible therapeutic role for such organisms. Recently, clinical trials have been conducted with living parasites to treat patients suffering from a number of immune diseases and, at least in some cases, administering immunomodulating helminths can indeed contribute to amelioration of the disease symptoms (Wammers et al., 2014; Evans and Mitre, 2015; Fleming and Weinstock, 2015; Maizels, 2016).

Beyond the effect of helminths on the host immune system, an associated question is the potential of parasites to adapt to the immune environment within a generation and/or among generations. Even though helminths have relatively long life cycles compared with viruses or bacteria, they still have much shorter generation times than their hosts, which give them an advantage in terms of adaptive potential. An interesting question is therefore how parasites respond when exposed to an up-regulated immune response. Serial passage is a powerful tool used to address experimental evolution of parasites (Ebert, 1998). Lineages of parasites can be maintained for generations in hosts expressing an up-regulated immune phenotype whereas control lines are maintained in control hosts. After a few generations of serial passages, the phenotypes of the selected parasites are compared between lines and between the ancestral and the evolved lines. Such an experimental evolution approach might be particularly relevant not only to address the fundamental question of parasite adaptation but also to predict the possible evolutionary trajectory of the therapeutic capacity of these organisms.

We investigated the microevolutionary response and adaptation to intestinal inflammation using the nematode *Heligmosomoides polygyrus* as a model organism. *Heligmosomoides polygyrus* is a gut nematode commonly used to study the molecular dialog between the parasite and the host immune system (e.g., Urban et al., 1991; Shea-Donohue et al., 2001; Ince et al., 2009; Hang et al., 2010; Reynolds et al., 2012). This species is a natural parasite of rodents with a direct life cycle, with the host becoming infected after ingestion of infective larvae. Upon infection, larvae penetrate into the wall of the small intestine and after a few days return into the intestinal lumen as adults and start releasing eggs that are shed into the external environment with the host feces.

Heligmosomoides polygyrus has been shown to interfere with the host immune response in several ways; it produces excretory/secretory molecules that provide protection for the host immune response, inhibiting the process of antigen presentation (Manoury et al., 2001; Sun et al., 2013) or by activating and stimulating the population of regulatory T (Treg) lymphocytes that dampen Th1 and Th2 responses (Grainger et al., 2010; McSorley et al., 2013). Due to this capacity to dampen the immune response, *H. polygyrus* has been shown to protect mice from the inflammatory symptoms of induced colitis (Elliott et al., 2004; Sutton et al., 2008; Hang et al., 2010; Blum et al., 2012; Donskow-Lysoniewska et al., 2012).

We conducted a serial passage experiment where *H. polygyrus* was maintained in hosts with induced colitis (inflammation of the colon due to the ingestion of dextran sulfate sodium (DSS)) or in control hosts. After nine generations of selection, worms from each selection regime were used to infect mice treated with DSS or control hosts. This allowed us to disentangle the effect of the current immune environment from the microevolutionary response to inflammation.

2. Materials and methods

2.1. 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 N7794) according to the national guidelines (Charte nationale portant sur l'éthique de l'expérimentation animale) on the use of animals for research purposes.

2.2. Mice and H. polygyrus selection regimes

BALB/c female mice were purchased from JanvierLABS (Laval, France) and housed (five individuals per cage, 18.5 × 38 × 22.5 cm, enriched with shelters) at the Université de Bourgogne, France. They were maintained under a constant temperature (24 °C) and photoperiod (12L:12D), and received food pellets and filtered tap water ad libitum.

Mice used for the serial passages were infected with 150 L3s of *H. polygyrus* in 0.1 ml of water, by oral gavage using a feeding needle on a 1 ml syringe. The original stock of parasites was maintained in B6CBAF1 female mice. Five mice were used for each selection regime per generation. The two selection regimes were: exposure to an inflammatory environment or a control environment. To this purpose, mice were given either a DSS solution in drinking water for 4.5 days (starting 2 days prior to *H. polygyrus* infection) or drinking water only. DSS is a complex branched glucan which induces colitis (Chassaing et al., 2014). We used a 1% DSS solution from generation 0 (G_0) to generation 3 (G_3) and then moved to a stronger selection regime (3.5% DSS) from G_4 to G_9.

We used 20 mice to assess the life history traits of the original stock of *H. polygyrus* (G_0), and 40 mice to assess the life history traits of the selected lines after nine generations (G_9). To this purpose, at G_0, 10 mice were exposed to DSS (1% for 4.5 days) and 10 mice were left as controls. Two days after the start of the DSS treatment, each mouse was infected with 150 L3 s from our original stock. We monitored parasites by fecal egg counts at days 9, 12, 16, 19, 23 and 26 p.i., as follows. Mice were transferred at 9 am into individual cages with a grid on a humidified towel paper at the bottom to prevent feces desiccation. Mice were left for 4 h in these cages and then put back in 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% of saturation) were added to allow eggs to float. After agitation, a fraction of this suspension was transferred into a McMaster chamber for the egg count. We performed two counts per sample and used the mean values (repeatability of egg count, R = 0.99, n = 34). A fecal egg count was expressed as the number of eggs per mg of feces. We also used these counts to compute overall egg production as the sum of individual fecal egg counts throughout the study.

At day 13 p.i., half of the mice were killed by cervical dislocation. The abdomen was immediately opened and the number of adult worms (and the number of female worms) in the intestinal lumen was counted. The other of mice in each group were euthanized at day 27 p.i., and the same procedure was used to count adult worms in these mice. Counting female worms allowed the calculation of per capita fecundity at days 12 and 26 p.i.

After nine generations of selection, parasites from each selection line (DSS and control) were used to infect 10 mice that were exposed to DSS (3% for 4.5 days starting 2 days prior to the infection) or left as controls. Therefore, we had four groups of mice (n = 10 mice per group) where the selection regime and the current environment were crossed in a factorial design. Parasite life history traits (fecal egg count, number of adult worms at days 13 and 27 p.

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