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Review Article

Interactions between the intestinal microbiome and helminth parasites

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SUMMARY

Throughout evolution, both helminths and bacteria have inhabited our intestines. As intestinal helminths and bacteria inhabit the same environmental niche, it is likely that these organisms interact with, and impact on, each other. In addition, intestinal helminths are well known to alter intestinal physiology, permeability, mucous secretion and the production of antimicrobial peptides – all of which may impact on bacterial survival and spatial organization. Yet despite rapid advances in our understanding of host–intestinal bacteria interactions, the impact of helminths on this relationship has remained largely unexplored. Moreover, although intestinal helminths are generally accepted to possess potent immuno-modulatory activity, it is unknown whether this capacity requires interactions with intestinal bacteria. We propose that this 'ménage à trois' situation is likely to have exerted a strong selective pressure on the development of our metabolic and immune systems. Whilst such pressures remain in developing countries, the eradication of helminths in industrialized countries has shifted this evolutionary balance, possibly underlying the increased development of chronic inflammatory diseases. Thus, helminth–bacteria interactions may represent a key determinant of healthy homoeostasis.

Keywords *helminths, intestinal bacteria, microbiota, nematodes*

INTRODUCTION

In most natural environments, the large majority of mammals harbour soil-transmitted helminths (STH) that often live as adults within the intestine for prolonged periods (1–2 years). Although these organisms have been eradicated to large extent within westernized human populations, those living within rural areas of developing countries continue to suffer from endemic infection rates. Indeed, recent estimates indicate that approximately 2 billion people, mainly children, currently suffer from infections with intestinal helminths (1). Evidence of heavy STH burdens can be found in the mummified remains of early hominids (2), and these organisms were recently described to represent the main selective force for the selection of human genes associated with autoimmunity (3). Although they rarely kill, STHs impact on human health through impaired nutrition leading to growth retardation, vitamin deficiencies and poor cognitive function, with some species additionally causing anaemia (4). STH are well known to suppress host immunity to establish chronic infections, and may impact on host responses against other pathogens or environmental antigens (5). Chronic intestinal helminth infection has been documented to lower the efficacy of vaccination, to increase susceptibility to co-infection and to attenuate the severity of allergic and autoimmune disorders in mice and man (6, 7).

All mammals harbour dense communities of non-pathogenic bacteria that live in harmony with their host and colonize the mucus membranes and skin soon after birth. In humans, the greatest density and complexity of bacteria are found within the intestine, with approximately 10^{12} organisms per gram of intestinal content represented by approximately 1000 different species (8). Accurate analysis of this complex bacterial community (also referred to as the microbiome) relies on molecular-based methods as many of the species cannot be cultured. To date, the large majority of studies utilize a technique involving sequencing of PCR-amplified bacterial 16S ribosomal RNA (rRNA) genes. These genes contain hypervariable regions that provide species-specific signature sequences. Sequences are

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organized into operational taxonomic units (OTUs) by alignment-based clustering (typically at a 3% distance level), and the represented bacterial species are predicted by alignment of the sequence against taxonomic databases. The total number of OTUs can also be used to measure bacterial diversity and to detect differences between bacterial communities from distinct samples.

There is a growing awareness that our microbiome participates in an intimate and complex dialogue with the host's immune and metabolic systems. Alterations to the microbiome have been associated with a number of chronic inflammatory diseases including obesity, inflammatory bowel disease**,** diabetes and allergy (9). A common theme arising from human microbiome studies is the finding that health can be linked to microbial diversity, with diseased states often associating with a less diverse bacterial community (and/or the selective outgrowth of specific species), whilst healthy states associate with increased bacterial diversity (10). To date, the major factors identified as important regulators of the microbiome include antibiotic use, diet, genetics and the presence of pathogens (9).

Our immune system has co-evolved together with large numbers of intestinal bacteria and the almost ubiquitous presence of STHs. Moreover, as many STHs inhabit the intestinal niche as adult worms, it is highly likely that these organisms impact on the microbiome. Thus, it is pertinent that we begin to view the intestine as a complex community in which the host, microbiome and STHs all interact. In the current review, we highlight existing reports describing helminth–bacteria interactions within the host intestine, with a specific focus on soil-transmitted helminths. We outline the current knowledge regarding such interactions, their potential implications for the host and the major challenges ahead.

BACTERIAL INTERACTIONS WITH PARASITIC HELMINTHS

Interactions between multiple organisms can be expected to exert strong pressure on co-evolutionary processes aimed at promoting mutual benefit, or to lead to the eradication of one partner. In a striking example of such interactions, Jaenike *et al.*, (11) reported that the presence of a maternally transmitted bacterium, *Spiroplasma*, in *Drosophilia neotestacea* protects flies against the sterilizing effects of a parasitic nematode. Although the exact mechanisms by which the bacterium protects against helminth infection remain unclear, experimental co-infection of flies with *Spiroplasma* and the helminth *Howardula aoronymphium* resulted in the recovery of stunted female worms which harboured a reduced number of eggs (11). In another recent study, Hayes *et al.*, (12) reported that successful establishment of the STH, *Trichuris muris,* in mice requires the presence of intestinal bacteria. Trichuris species inhabit the caecum and proximal colon of a diverse range of mammalian hosts, with infection occurring following the ingestion of an embryonated egg. The authors showed that bacterial association with the egg polar plug provided an essential cue that triggered the emergence of infective larvae following its arrival into the bacteria-rich caecum of mice (12). Whilst the first study reports an example of how bacteria-mediated protection against a helminth can benefit the host (by preventing sterility), the latter study demonstrates an exploitation of intestinal bacterial communities by a parasitic helminth that favours the worm.

Other examples of helminth–microbiome interactions can be found in older studies investigating the development and fecundity of helminths in animals raised under axenic (germ-free) conditions in which mice lack all intestinal bacteria. Wescott *et al.*, infected germ-free mice with the natural murine nematode, *Heligmosomoides polygyrus bakeri* (at that time known as *Nematospiroides dubius*), and reported that high dose infection caused mortality in germ-free, but not conventionally raised, animals (13). Similarly, a greater number of adult worms could be recovered from conventional compared to germ-free or gnotobiotic mice (mono-associated with a *Lactobacillus* species) following low-dose *H. polygyrus bakeri* infection (14). Comparable results were obtained in mice utilizing other helminth species, including *Nippostrongylus brasiliensis* (15) and *Trichinella spiralis* (16), and in chickens infected with *Ascaridia galli* (17). Together, these findings suggest that intestinal bacteria promote infection by parasitic helminths, a finding supported by a recent study showing that treatment of conventional BALB/c mice with *Lactobacilli taiwanensis* results in a greater establishment of *H. polygyrus bakeri* (18). In this study, the authors chose to administer *L. taiwanensis* after they identified an outgrowth of this bacterial species following infection of genetically susceptible C57BL/6, but not resistant BALB/c, mice with *H. polygyrus bakeri*. Thus, their findings may indicate a co-evolutionary relationship in which the bacterium and helminth co-operate to promote the establishment of both organisms within the intestine of their mammalian host. By contrast, studies investigating the protective potential of probiotics have yielded data showing that oral treatment of mice with bacteria can protect against, rather than promote, helminth infection. In these studies, *Lactobacillus casei* was shown to reduce adult worm burdens of *T. spiralis* (19), whilst *Bifdobacterium animalis* strain 04450B provided protection against the helminth *Strongyloides venezuelensis* (20). Thus, the outcome

of bacteria–helminth interactions is likely to differ depending on the exact species involved.

Unfortunately, none of these studies provide evidence for whether direct interactions between bacteria and helminths are occurring, or whether such interactions occur indirectly via the mammalian host. It is well established that the microbiome can modulate host immunity, raising the possibility that such changes may also impact on the host immune response against STHs. The presence of intestinal bacteria is required for the normal development of the immune system, as evidenced in germ-free mice which exhibit a reduced size and abnormal structure of lymphoid tissues at both mucosal and systemic sites (21). Germ-free mice also exhibit decreased IgA levels, but heightened production of IgE (22, 23), and increased numbers of immune cells associated with allergic responses including basophils (22, 24) and mucosa-associated invariant natural killer T cells (25). In the large intestine, bacterial communities are also known to drive the development of inducible regulatory T cells (26). Many of these components of the immune system are also mobilized in response to STH infection; thus, it will be important to determine how bacterial-driven immune modulation impacts on anti-helminth immunity using experimental models employing germ-free or antibiotic treated mice.

MODULATION OF INTESTINAL BACTERIAL COMMUNITIES BY HELMINTHS

As mentioned previously, a diverse or complex intestinal bacterial community is thought to be necessary for a healthy immune and metabolic system. This may result from more diverse bacterial communities harbouring greater gene content, or from diverse communities being more stable in the face of transient disturbances (such as antibiotic use). Interestingly, microbiome studies comparing individuals from different countries have indicated a greater microbial diversity in rural populations living in developing nations as compared to urbanized populations living in developed nations (27). These differences have mainly been attributed to differences in diet; however, populations living in rural areas of developing nations without adequate access to sanitation often exhibit a high prevalence of helminth infection, whilst these organisms have been eradicated in most developed nations. To date, relatively few studies have specifically addressed the impact of helminth infection on the microbiome. The first such study compared bacterial communities in stool samples of school children living in rural Ecuador. The study separated children into three groups, those with no obvious helminth infection, those infected with *T. trichiura* and those co-infected with *T. trichiura* and *A. lumbricoides* (28). 16s rRNA sequencing revealed no obvious differences in bacterial community composition of noninfected children or children harbouring *T. trichiura*. However, a decreased abundance of bacterial species belonging to the Clostridia class of Firmicutes, as well as a reduction in overall bacterial diversity, was noted for children co-infected with *T. trichiura* and *A. lumbricoides* (28). In a second study, Lee *et al.*, (29) compared faecal stool samples from helminth-infected or noninfected individuals living in rural Malaysia. The authors reported a significant increase in bacterial diversity amongst individuals infected with any helminth species, and an increased abundance of bacterial species belonging to the Paraprevotellaceae family in those individuals infected only with the helminth *T. trichiura*. Finally, a third study reported the impact of experimental infection with the helminth *Necator americanus* on intestinal bacterial communities in patients suffering from coeliac disease (30). The authors compared stool samples from eight coeliac patients (all on a long-term gluten-free diet) both before and after infection with a low dose of *N. americanus* (30). No clear impact on microbial community structure, diversity or relative abundance was observed 8 weeks following helminth infection; however, there was a trend towards increased bacterial richness in infected individuals (30). Overall, these studies indicate that helminth infection may promote bacterial diversity; however, strong conclusions are limited both by the small number of studies and by the relatively small sample size employed within each study. Further studies will be required to clarify the impact of human helminth infection on the microbiome, and these should ideally include larger numbers of individuals, comparisons to dietary metadata and more detailed information on the presence or absence of other common intestinal pathogens (such as pathogenic bacteria, viruses or protozoa).

A more comprehensive picture of helminth-induced alterations to the microbiome is beginning to emerge in experimental animal models. The murine helminth *H. polygyrus bakeri* was reported in two separate experiments to increase the abundance of species belonging to the Lactobacillaceae family within the small intestine (where the worm resides) (31), whilst no major impact on caecal bacterial communities was noted (31). Two further studies, which both employed a PCR-based approach targeting selected bacterial families, confirmed a trend for higher *Lactobacilli* loads in the small intestine of *H. polygyrus bakeri*-infected mice (18, 31). Rausch *et al.,* (32) additionally observed an impact of *H. polygyrus bakeri* infection on caecal bacteria despite the worm residing in the upper small intestine. In their study, infection was observed to result in a significant increase in the number of bacterial species belonging to γ-Proteobacteria/

Enterobacteriaceae and Bacteroides families present in the caecum. However, as a PCR-based approached was used, the relative abundance of these species within the intestine, and possible alterations to other bacterial species not targeted by the primers used, cannot easily be determined. Of note, these authors also reported that similar changes to caecal bacterial species belonging to the Enterobacteriaceae family were found in infected wild-type or IL-4Ra-deficient mice, indicating that helminth-induced bacterial disturbances may occur independently of the type 2 immune response (32). Similar observations have also been made with another helminth species, *Trichuris muris*, whereby chronic infection of wild-type mice caused a drop in bacterial diversity as well as an increased relative abundance of *Lactobacilli* (23).

In pigs, *T. suis* infection was shown to increase the abundance of bacterial species belonging to the phyla Proteobacteria and Deferribacteres, with the increase in Proteobacteria species resulting almost solely from an outgrowth in the mucus dwelling bacteria, *Mucispirillum* (33). This, and a second a study from the same group, additionally reported a reduced abundance of bacterial species belonging to the phylum Ruminococcus in infected pigs (33, 34). Surprisingly, changes in the microbiota were noted to occur independently of worm burdens, indicating that microbial changes may result from the initial infection event and remain even after the worm burden is reduced by the host (34). This is an important finding as human studies often rely on a snapshot of worm infection status, but may include individuals who were previously exposed to helminths but who had either cleared all of the worms, or harboured worm burdens at levels below the detection limit of current techniques.

For the large majority of reports, notable variations in sample collection and analysis technique preclude meaningful comparisons, even where the same helminth species was investigated. Collectively however, these studies do reveal a likely role for STHs in modulating the microbiome. The future challenges will be to assess the real impact of STH infection on microbial communities using standardized approaches and to determine the mechanisms by which this may occur. In this regard, intestinal helminths constantly secrete a large array of products that could potentially modulate the growth or metabolism of resident microbial communities. Alternatively, they may compete directly with bacteria for energy-rich nutrients or essential minerals. STH infection can also have dramatic impacts on intestinal physiology, including increased fluid secretion, altered mucous production and the infiltration of host immune cells (35) – all of which are likely to impact on bacterial communities via alterations to their habitat. Alterations in mucous alone is likely to have dramatic consequences for bacterial growth and metabolism as many species use the mucous to anchor within the intestine, or as an energy source (36). To address these possibilities, carefully controlled studies comparing the impact of STH infection on bacterial communities in experimental animals will be necessary. Such studies would allow a close analysis of the bacterial communities present in the lumen or mucous layer of the small and large intestine of animals exposed to defined numbers of infective larvae. Ideally, they would additionally investigate whether STH infection altered the ability of the host to prevent bacterial translocation across the intestinal epithelium. Murine studies should prove especially informative, as they would allow researchers to determine the role of helminth-elicited immunity in altering the microbiome using the wide array of available genetically modified murine strains in which specific immune compartments are altered.

THE IMPORTANCE OF HELMINTH–MICROBIOME INTERACTIONS FOR THE HOST

An elegant study of how helminth–microbe interactions can impact on the host is found in the example of lymphatic filariasis and onchocerciasis. Filarial helminths causing these diseases carry a bacterial endosymbiont, *Wolbachia*, which contributes greatly to the inflammation that causes pathological reactions following infection (37). As discussed, the microbiome is well known to impact on the immune status of the host, and alterations to the microbiome have been associated with a number of chronic inflammatory diseases including obesity, inflammatory bowel disease**,** diabetes and allergy (9). Although it is not always easy to determine whether the relationship between bacterial dysbiosis and disease states is causal or correlative, several recent studies indicate that the microbiome does play a causative role. These include experimental transplantation experiments in which the microbiota of individuals suffering from obesity or inflammatory bowel disease was transplanted into germ-free recipients resulting in disease transfer (38, 39). In addition, prevention of disease by the microbiota has been demonstrated for type 1 diabetes (40), and inflammatory arthritis (41), again in studies using germ-free mice. Our own laboratory has contributed to this work by recently demonstrating that germ-free mice exhibit exaggerated allergic asthma following intranasal allergen challenge (22). Lastly, faecal transplantation from healthy to diseased humans has been successfully used as a treatment for inflammatory bowel disease and *Clostridium difficile*-associated diarrhoea (42, 43). One might therefore expect that helminth-induced alterations to intestinal bacterial communities are likely to result in

alterations to the severity or prevalence of immune and metabolic diseases in their mammalian hosts.

Interestingly, the absence of intestinal helminths has been postulated to underlie the increasing prevalence of allergic and autoimmune diseases within westernized societies (7), and helminth-based therapeutics, including infection with the porcine whipworm, *T. suis*, or with *N. americanus,* have been trialed in allergy and inflammatory bowel disease (44). Thus, it would be of tremendous interest to investigate whether the immune-modulatory potential of helminths involves, at least in part, alterations to the microbiome. Recent studies indicate that this may indeed be the case. Following on from studies reporting that helminth infection may provide protection against *Helcobacteri*-induced gastric adenocarcinomas (45) and that the development of lesions required bacterial invasion of the gastric mucosa (46), Whary *et al.,* (47) performed experimental studies investigating the impact of *H. polygyrus bakeri* infection on *H. polyri*-induced gastric lesions in susceptible male mice (which were transgenic for INS-GAS). Helminth infection prevented increased bacterial colonization of the stomach and reduced the number of neoplastic lesions (47). In Macaques, infection with the helminth *Trichuris trichiura* was reported to improve clinical symptoms in monkeys suffering from idiopathic chronic diarrhoea (ICD), a leading cause of morbidity amongst rhesus monkeys kept in captivity. Helminth-mediated protection against ICD was associated with a mucosal type 2 immune response, reduced bacterial attachment to the intestinal mucosa and alterations to bacterial community structure including reductions in bacterial species belonging to the phylum Cyanobacteria and an expansion of species belonging to Tenericutes (48). Inflammatory bowel disease is postulated to be driven by aberrant responses to intestinal bacteria (49) and is often associated with a reduced microbial diversity and increased attachment of bacteria to the mucosa (50, 51). Thus, the ability of *T. trichiura* to reduce bacterial mucosal attachment in ICD monkeys is likely to be a key component of its ability to ameliorate disease.

Intestinal bacteria are also thought to impact on, and to share, metabolic pathways with their mammalian hosts. Metabolic and immune pathways are linked at many levels, including alterations in endocrine signalling pathways and the direct sensing of metabolites by host immune cells. Short-chain fatty acids (SCFAs) represent just one pathway of bacteria–host co-metabolism and are end products of fermentative metabolism of complex plant polysaccharides by intestinal bacteria. Bacterial-derived SCFAs are well acknowledged to impact on host health by modulating both metabolic status (52) and immune function (53, 54). Although often overlooked, many helminth parasites also impact on host metabolism (55) and can produce the SCFA acetate (56). Thus, helminths are likely to represent a key part of the intestinal microbe–host metabolic axis. In this regard, it is interesting to note that murine infection with *H. polygyrus bakeri* has been noted to result in increased numbers of colonic IL-10 producing regulatory T cells, which in turn provide protection against inflammatory colitis (57). Colonic regulatory T cells are widely regarded to arise in response to intestinal bacteria (26, 58), a finding that has recently been linked to the activity of bacteria-derived SCFAs (53). Thus, it would be interesting to explore the role of intestinal bacteria in promoting anti-inflammatory colonic regulatory T cells following *H. polygyrus bakeri* infection. Given that one of the major pathways in which intestinal bacterial communities communicate with the host is through the conversion of dietary components into metabolites (59), future studies investigating the impact of helminth infection on bacterial communities would ideally include an analysis of metabolic pathways. This can be achieved using a shot-gun-, rather than PCR-, based approach to sequence intestinal contents. This approach allows the analysis of total bacterial gene content, and bacterial metabolic function can then be inferred from gene content using existing databases. Where necessary, such studies could be further complemented by meta-transcriptomics or meta-proteonomic approaches to investigate which microbial metabolic processes are active within the host.

CONCLUSIONS

Recent studies in both humans and animals indicate that helminth infection can impact on the intestinal microbiome, whilst other studies demonstrate an intimate relationship between bacteria and helminths resulting in an impact on worm number and/or fitness. These studies indicate that future work investigating intestinal bacterial communities in areas endemic for helminth infection – or investigating the impact of helminth infection on host health – should consider these interactions as forming only one part of a more complex ecosystem in which helminths, bacteria and the host are all interconnected. As with any ecosystem, external disturbances (including the removal of helminths or the use of antibiotics) are likely to impact on all components within the system and will require a process of recovery or adaptation. Such a view also implies that the use of adaptive management schemes – akin to those used for the conservation of natural resources within ecological ecosystems – will be vital to the long-term maintenance of human health.

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DISCLOSURES

The authors have no potential conflict of interest to disclose.

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