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Cohabitation in the Intestine: Interactions among Helminth Parasites, Bacterial Microbiota, and Host Immunity

Lisa A. Reynolds,* B. Brett Finlay,*‡ and Rick M. Maizels§

Both intestinal helminth parasites and certain bacterial microbiota species have been credited with strong immunomodulatory effects. Recent studies reported that the presence of helminth infection alters the composition of the bacterial intestinal microbiota and, conversely, that the presence and composition of the bacterial microbiota affect helminth colonization and persistence within mammalian hosts. This article reviews recent findings on these reciprocal relationships, in both human populations and mouse models, at the level of potential mechanistic pathways and the implications these bear for immunomodulatory effects on allergic and autoimmune disorders. Understanding the multidirectional complex interactions among intestinal microbes, helminth parasites, and the host immune system allows for a more holistic approach when using probiotics, prebiotics, synbiotics, antibiotics, and anthelmintics, as well as when designing treatments for autoimmune and allergic conditions. The Journal of Immunology, 2015, 195: 4059–4066.

The mammalian immune system has evolved to cope with immense microbial presence, including some dangerous, some harmless, and some beneficial microbes (1), as well as in conjunction with macrobionts, such as helminth parasites (2). In each case, host immunity has to make the correct judgment about whether to reject or accept the new species and, if the latter, how to control it. In parallel, incoming organisms have evolved to maximize their chances of acceptance, through immune evasion, mimicry, and induction of host immunoregulatory pathways. Thus, although commensal bacteria and multicellular helminths occupy very different taxonomic space, they have both responded to evolutionary forces by developing similar strategies of modulating host immunity. Moreover, it is apparent that these different kingdoms of life have developed a surprising degree of dialogue with a common agenda of establishing a new homeostasis in the host intestinal tract (3, 4).

The parallel agendas of bacterial microbes and intestinal helminths include dampening or deceiving host immunity to permit their survival, even though bacteria and helminths need to suppress very different Th1/17- and Th2-dominated effector mechanisms, respectively. Common strategies include the induction of suppressive regulatory T cells (Tregs) by a range of bacteria, including Bacteroides fragilis (5), Bifidobacterium infantis (6), Clostridium spp. (7–9), and Lactobacillus spp. (10–13), as well as by intestinal nematode parasites, such as Heligmosomoides polygyrus (14) and Strongyloides ratti (15). Interestingly, activation of Tregs appears to be a widespread feature of both microbiota colonization (16, 17) and helminth parasite infection (18) (Fig. 1).

Expansion of Treg activity may underpin an additional feature shared among many helminths and microbiota species: the systemic muting of the immune response so that reactivity to bystander Ags, such as allergens and autoantigens, is inhibited. The parallels were not immediately articulated and, indeed, separate models emerged of helminth-mediated (19) and microbial-mediated (20) protection against allergy, before more recently coalescing. These similarities are illustrated by the fact that both H. polygyrus (21, 22) and Lactobacillus spp. (12) in the intestinal tract can block the development of allergic reactivity in the airways of mice, whereas heightened susceptibility to allergy development in humans and in animal allergy models can result from either antibiotic or anthelmintic treatment (23–25). Likewise, both commensals (26, 27) and helminths (28) can ameliorate autoimmune and colitic disease. It will be interesting to follow these parallels as further systemic effects of intestinal colonization come to light, including changes in metabolism, obesity, and behavior (29–31).

Given the relatively unexplored theme of bacterial–parasite interactions within the mammalian host and the emerging therapeutic potential of both bacterial microbiota species (32) supported by grants from the Rainin Foundation (Grant 12-H4) and the Wellcome Trust (Grant 106122).

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Abbreviations used in this article: DC, dendritic cell; ES, excretory/secretory; HES, ES product of H. polygyrus; SCFA, short-chain fatty acid.

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and parasitic helminths (33), it is now essential to understand the multilateral interactions between these organisms and the host immune system. In this review, we discuss the experimental evidence regarding these relationships and examine to what extent the reported immunomodulatory effects of helminths can be attributed to a modulation of microbiota composition or function.

Helminth infection modulates bacterial microbiota composition and function

Controlled laboratory animal experiments clearly demonstrated that infection with helminth parasites results in substantial shifts in the intestinal microbiota species composition. Chronic *H. polygyrus* infection in the duodenum of mice results in an increased abundance of Lactobacillaceae and Enterobacteriaceae species in the small intestine (34–36). Similarly, a chronic infection with the mouse whipworm *Trichuris muris*, which colonizes the cecum, leads to a reduced diversity of fecal bacterial species, particularly within the phylum Bacteroidetes, as well as an increase in the abundance of Lactobacillaceae family members (37, 38). Rats infected with the tapeworm *Hymenolepis diminuta* had an altered community structure compared with uninfected animals that involved ∼20% of their total cecal bacterial microbiota, with a general shift in abundance from class Bacilli to the class Clostridia in helminth-infected rats (39). Microbiota changes following helminth infection correlate with worm burdens (36, 40) but revert to normal following drug clearance of helminths, indicating that the continuing presence of parasites is required for sustained changes to the bacterial microbiota (38). In wild mice (*Apodemus flavicollis*), around half of animals sampled were simultaneously infected with more than...
one helminth species, most commonly a combination of *H. polygyrus*, *Syphacia* spp. (pinworm), and *Hymenolepis* spp. (tapeworm) (41). Helminth infections correlated with heightened bacterial microbiota diversity, with the presence of each helminth being associated with specific shifts in microbiota species composition or abundance (41).

Very recent findings indicate that helminth infection can also modify host metabolism, with ensuing implications for immune modulation. Thus, experimental *T. muris* infection in mice reduced a large number of metabolomic products, as measured in the feces, including vitamin D2/D3 derivatives, dietary plant-derived carbohydrates, and amino acid synthesis intermediates (38); hamsters infected with the human hookworm *Necator americanus* similarly showed extensively altered urinary metabolite levels that could be explained by changes in the intestinal microflora (42). Infection of pigs with the related porcine whipworm *Trichuris suis*, which also alters the composition of the colonic microbiota, is again accompanied by a metabolic shift, with infection resulting in reduced cofactors for carbohydrate metabolism and amino acid biosynthesis (40, 43). Such metabolomic alterations following helminth infection may result from microbiota compositional changes, altered intestinal absorption of dietary products, or direct production of metabolites by helminth parasites (38).

In human populations, studies on the influence of helminth infection on microbiota composition and function have only recently commenced. In a cohort of Zimbabwean children, those positive for *Schistosoma hematobium* infection were found to have a significantly higher fecal abundance of several operational taxonomic units from within the genus *Prevetella* (44). In these subjects, praziquantel-induced helminth clearance did not revert the microbiome composition, suggesting that childhood helminth exposure may have long-term effects on microbiota community structure (44). In a Malaysian population, the fecal microbiota of individuals colonized by at least one helminth parasite (*Trichuris* spp., *Ascaris* spp., or hookworms) harbored a more diverse community compared with individuals free from helminth infection (45). However, less marked differences emerged from a study of school-age children in Ecuador with similar helminths (46) or from eight human volunteers experimentally infected with *N. americanus* (47).

It should be noted that populations with a high prevalence of helminth parasites are also very distinct in diet, lifestyle, and host genetics from those in major industrialized societies and appear to carry markedly different sets of intestinal microbes (48). Interpretation of data is likely to be further confounded by variable infection intensities in natural helminth infections. These human studies are also restricted to fecal analyses, which do not accurately reflect local microbiota shifts that may occur, for example, postinfection with small intestine dwelling hookworms (e.g., *N. americanus*) and roundworm (*Ascaris lumbricoides*).

**Helminth infections: resetting immune homeostasis and impact on microbiota**

A characteristic feature of helminth infection is the elicitation of a type 2 immune response, alongside a regulatory response, especially in the setting of chronic, asymptomatic infection (2). Given the immune system’s role in regulating and containing the intestinal microbiota population (1), it seems likely that disruption and rebalancing of immune homeostasis can result in functional shifts in microbial composition. Interestingly, such changes to the set points can be observed through both innate and adaptive pathways (Fig. 2).

A significant effect of helminths on innate interactions with the microbiota may be to alter the production of antimicrobial peptides in the intestinal tract. BALB/c mice, which mount a Th2-polarized immune response following *T. muris* infection, showed increased expression of the antimicrobial peptide angiogenin 4 in colonic goblet cells after *T. muris* infection (49). Furthermore, *H. polygyrus* infection increased expression levels of the antimicrobial C-type lectin RegIIIγ in the cecum of mice (50). Such alterations in antimicrobial peptide secretion leading to microbiota compositional shifts following helminth infection may be evoked by specific products released by helminths (termed excretory/secretory [ES] products) acting on intestinal epithelial cells. Consistent with this is a report that the broad microbiota compositional changes caused by *H. polygyrus* infection in mice were independent of IL-4Rx signaling and Th2 induction (35).

An altered physical microenvironment elicited by helminth infection, including epithelial barrier disruption and the stimulation of mucus production, may also select for the outgrowth of specific species within the microbiota (43). Changes to the intestinal mucus layer include a switch from Muc2 to Muc5AC following *T. muris* infection (51) and more subtle changes to the glycosylation patterns of mucins (which impact upon viscosity) following infection of rats with the rodent helminth parasite *Nippostrongylus brasiliensis* (52). Most significantly, perhaps, the IL-13/IL-22–dependent hyperproliferation of goblet cells and overproduction of mucus following helminth infection (53) are likely to substantially alter the ability of different bacterial species to remain in the intestinal tract.

TLR interactions are central to the maintenance of host–microbiota homeostasis (54), and interference with TLR or other pattern recognition receptor signaling may be a mechanism by which the presence of helminths alters microbiota composition. There is evidence that helminth infection can alter expression levels of TLRs (55, 56) and modulate downstream signaling following TLR stimulation (28, 57–59). Within the intestinal setting, infection of rats with *H. diminuta* increases expression of TLR2 and TLR4 (60), whereas *H. polygyrus* infection induces TLR4 expression specifically on small intestinal lamina propria T cells (61), which may be stimulated through the increased exposure of host immune cells to microbiota ligands during helminth infection.

As well as altering TLR expression levels, it is well documented that helminth ES products can modulate inflammatory responses from dendritic cells (DCs) and macrophages following stimulation with TLR ligands (62). For example, a fatty acid–binding protein from the human and animal parasitic trematode *Fasciola hepatica* (Fh12) can suppress IL-12p35, TNF-α, IL-6, and IL-1β production from bone marrow–derived macrophages in response to LPS stimulation (63), and the ES products of *H. polygyrus* (HES) can suppress IL-12p70 and IL-10 production in response to CpG stimulation of bone marrow–derived DCs (64). Interestingly, ES products from the whipworm *T. suis* downregulate DC TLR...
responses but interacts with C-type lectin receptors though specific glycan moieties (65). The functional role of these modulatory responses in the intestinal setting is not clear; however, these pathways may be important in situations where helminth infection promotes host tolerance against specific groups within the bacterial microbiota.

Modulation of microbiota populations through the adaptive, Ag-specific arm of the immune system can also take place. For example, microbiota-specific T cells are generated following epithelial barrier breach induced either by dextran sodium sulfate administration or by acute infection with the protozoan parasite *Toxoplasma gondii* (66). Intestinal helminths could likewise boost the T cell response to microbial Ags, although in other contexts certain helminth species effectively downregulate the host T cell compartment (67) to establish a more tolerogenic environment.

An additional component aiding in containment of the intestinal microbiota is the production of mucosal IgA by lamina propria plasma cells, which is stimulated by the presence of the microbiota itself (68). Surprisingly, although robust parasite-specific IgA responses are elicited in helminth infections, these Abs have only a limited role in protective antiparasite immunity (69, 70). However, it is possible that helminth infection modulates the generation of microbe-specific IgA responses, as indeed is reported in the suppression of cholera toxin IgA Abs in patients coinfected with helminths and *Vibrio cholerae* (71).

A fascinating study of the adaptive Th2 response that may modulate both microbial populations and host pathology concerns the treatment of spontaneous idiopathic chronic diarrhea among captive rhesus macaques. The experimental administration of *Trichuris trichiura* ova improved disease symptoms (measured by an increased fecal consistency and weight gain) in four of five animals, despite the lack of establishment of a chronic infection with *T. trichiura* (72). In these animals, a higher frequency of IL-4–producing CD4+ T cells was detected in colonic biopsies taken after, compared with before, helminth exposure (72). Additionally, following helminth exposure, the total load of several bacterial taxa detected in colon biopsies was reduced alongside a heightened diversity of bacterial species (72). A local colonic Th2 response induced by *T. trichiura* exposure may have promoted mucus production and epithelial turnover that were sufficient to reduce the association of bacterial microbiota species with the colonic mucosa, recovering intestinal homeostasis (73).

Independently of these immunological pathways, there are, of course, likely to be direct interactions between helminths and microbes, as suggested by the identification of an anti-bacterial peptide from the pig roundworm *Ascaris suum* (74) and the finding that HES contains at least eight lysosome homologs with potential antimicrobial effects (75). Furthermore, the ES products of *T. suis* had antibiotic activity in vitro, although the active principle was not identified (76). The extent to which these effects functionally alter the microbial composition in situ remains to be tested.

**Impact of helminths on infection with enteric bacterial pathogens**

In addition to impacting the composition and function of the commensal and symbiotic bacterial microbiota species, helminth infection can alter the host response to infection with pathogenic bacterial species. *H. polygyrus* or *N. brasiliensis* coinfection in mice impairs the clearance of *Salmonella enterica* serovar Typhimurium (S. Typhimurium) compared with *S. Typhimurium* infection alone, resulting in increased mortality, more pronounced edema of intestinal tissue, further epithelial erosions, and increased thickening of the gut wall (50, 77). Similarly, mice infected with *H. polygyrus* prior to *Citrobacter rodentium* infection show higher bacterial colonization levels and greater *C. rodentium*–induced pathology than mice that were singly infected, as measured by increased weight loss, epithelial cell hyperplasia, inflammatory cell infiltration, thickening of the gut wall, and higher incidences of anal prolapse and mortality (78). In this experimental system, the effect of helminth coinfecion was shown to be dependent on the type 2 immune response induced by *H. polygyrus*, because coinfected STAT6-deficient mice did not exhibit exaggerated disease severity (78). This may be due, in part, to a helminth-induced type 2 response repressing effector IFN-γ responses toward *C. rodentium* (78), although multiple additional parallel mechanisms likely contribute to the exaggerated pathology in helminth-coinfected mice.

**Feedback from bacterial microbiota to modulate helminth colonization and persistence**

The first striking example of how helminth parasites require the presence of the microbiota to successfully colonize...
mammals came from the observation that *T. muris* eggs, which hatch in the large intestine of their hosts after ingestion, fail to do so without signals from the bacterial microbiota (79). *T. muris* likely uses the high density of microbes in the large intestine as an environmental cue to trigger hatching in the correct location for its larvae to emerge. The requirement for the microbiota seems to be common among helminth parasites, because *H. polygyrus* is less able to form persistent infections in mice lacking a microbiota (germ free) compared with conventionally raised mice (80–82). This is particularly striking because germ-free mice are generally more susceptible to infections with bacterial or viral pathogens (83). Unlike *T. muris*, *H. polygyrus* eggs hatch in the external environment, and infective larvae are ingested (84); thus, additional mechanisms must underlie how the presence of the host microbiota benefits *H. polygyrus* survival within the host. It is possible that the failure of *H. polygyrus* to chronically infect germ-free mice results from morphological abnormalities along the intestinal tract of germ-free animals, such as an altered villous length (83); additionally, recent evidence suggests that, in conventionally raised mice, the composition of species within the microbiota can alter susceptibility to helminths (Fig. 2).

Treating mice with a low-dose antibiotic to modify the composition of their intestinal microbiota without significantly reducing the total load of bacteria is sufficient to alter susceptibility to infection with *H. polygyrus* (36). The abundance of *Lactobacillus* spp. in the duodenum was shown to positively correlate with *H. polygyrus* adult worm numbers 28 d postinfection and, importantly, experimental administration of the single commensal species *Lactobacillus taiwanensis* was sufficient to prolong the persistence of an *H. polygyrus* infection (36). That a chronic *H. polygyrus* infection results in *Lactobacillus* spp. expansion and that a *Lactobacillus* species is able to promote *H. polygyrus* infection points to mutually beneficial relationships between helminths and select bacterial species within the mammalian host (36). Similarly, the administration of live or dead *Lactobacillus casei* to mice was shown to enhance susceptibility to *T. muris* (85), and given that the abundance of *Lactobacillaceae* family members increases following *T. muris* infection, the possibility is raised that multiple helminth species have evolved to select for the expansion of bacterial species that promote their own persistence (37, 38).

A type 2 immune response is required for expulsion of helminths (84); thus, the presence of certain bacterial species within the microbiota may aid helminth persistence through inhibiting type 2 immunity. *L. casei* administration inhibited Th2 cytokine production in the mesenteric lymph nodes and Peyer’s patches of *T. muris*-infected mice (85), and *L. taiwanensis* administration resulted in an increased frequency of Tregs in mesenteric lymph nodes and Peyer’s patch tissue (36), although whether these are the primary mechanisms by which these *Lactobacillus* spp. promote susceptibility to helminth infection remains to be determined. The presence of a specific pathogen–free microbiota can stimulate the induction of RORγt Tregs in the intestinal lamina propria, and mice generated to specifically lack RORγt Tregs showed heightened frequencies of GATA3+ (Foxp3+) CD4+ T cells in their small intestinal lamina propria and were rendered more resistant to *H. polygyrus* infection (86).

If microbiota-specific responses are generated following epithelial barrier breach (66) during helminth infection, it may reduce the capacity of the host immune system to respond to helminth Ags. Additionally, microbiota compositional differences induced by helminths may lead to an altered metabolomic profile within the intestine, which has the potential to modulate the function of immune cells (87), conceivably reducing the capacity of the host to mount an effective parasite-clearing response.

Perhaps the most central mechanism through which the microbiota influence helminth infections is the ubiquitous TLR signaling pathway. Certainly, mice lacking the TLR adaptor protein MyD88 are better able to control *H. polygyrus* and *T. muris* infections than are MyD88-sufficient mice (88, 89). In both models, loss of MyD88 signaling resulted in greater Th2 cytokine release following helminth infection (88, 89). MyD88 mediates signaling through TLRs, but it also mediates signaling of IL-1 family members, including IL-1α, IL-1β, and IL-18 (90); thus, it is possible that a lack of helminth chronicity in MyD88-deficient animals is due to a loss of one or a combination of these signals. In the absence of TLR4 specifically, *T. muris* failed to maintain a chronic infection (89); however, loss of TLR4 alone did not affect *H. polygyrus* colonization (88), raising the possibility that, during *H. polygyrus* infection, redundant signaling through other TLRs or MyD88-dependent pathways maintains susceptibility to this parasite.

A further nexus of helminths, bacteria, and TLR signaling emerged from studies of mice treated with an antibiotic mixture during infection with *Schistosoma mansoni*; although parasites establish in the mesenteric vasculature rather than the intestinal tract itself, they release eggs that traverse the mucosal epithelium to enter the lumen. Antibiotic treatment significantly reduced the consequent granulomatous pathology in the intestinal mucosa, a reaction that was shown previously to require MyD88 signaling (91), but it also reduced egg egress into the feces; hence, optimal transmission by *S. mansoni* appears to require costimulation by the microbiota in the intestine (92).

**Immunomodulation during helminth infection: through parasites or microbes?**

Both helminth parasites and the bacterial microbiota are widely credited with immunomodulatory abilities (28, 93), leading to the question of whether the anti-inflammatory effects of helminth infection are due, at least in part, to changes in microbiota composition or function. Many soluble ES products released by helminths are able to ameliorate disease severity in mouse models of inflammation without the presence of active infection (28, 94–97); although it seems unlikely that each of these helminth ES products operate solely through modifying the host microbiota, the degree to which they modulate intestinal microbial biology has yet to be explored.

A key pathway contributing to the immunomodulatory abilities of helminth parasites, particularly in the context of suppression of allergic airway diseases, is the generation of Tregs (14, 21). Foxp3 expression in naive CD4+ T cells can be induced by exposure to HES, through a TGF-β-dependent pathway (14). A parallel induction of Tregs was described for many microbiota species (5, 7, 8, 10, 12, 13), including...
a mixture of several Clostridia spp., which are able to stimulate TGF-β1 production from human and mouse intestinal epithelial cell lines (8). Metabolites generated by the microbiota can also affect T cell differentiation in the intestine; the short-chain fatty acids (SCFAs) acetate, butyrate, and propionate can potentiate Treg generation and IL-10 production from Tregs in the periphery (98–100), which is notable because increased circulating SCFA levels are protective in a mouse model of allergic airway disease (101). Interestingly, parasitic helminths are also known to generate acetate (102), opening the possibility of another common pathway shared by microbiota and helminths. Given that helminth infection shifts the bacterial microbiota composition, and both helminths and the bacterial microbiota can exploit host pathways to generate intestinal Tregs (Fig. 1), it will be important to dissect the relative contributions of helminth product–elicited and microbiota-elicited Tregs during the dampening of allergic inflammation during helminth infection.

Conclusions

Microbes and helminths have coevolved within the mammalian host, and examples of their mutualism and the synergistic pathways by which they cause host immunomodulation to promote their own survival are beginning to emerge (Fig. 1). It is interesting to note that the distinction between symbiotic or commensal microbiota species and parasitic or pathogenic organisms plays through to important differences in their life strategies: to a large extent, parasitic or pathogenic organisms exploit the commensal or symbiotic microbiota species and both helminths and the bacterial microbiota can exploit host pathways to generate intestinal Tregs (Fig. 1), it will be important to dissect the relative contributions of helminth product–elicited and microbiota-elicited Tregs during the dampening of allergic inflammation during helminth infection.

Disclosures

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