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Parasitic Protozoa and Interactions with the Host Intestinal Microbiota

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ABSTRACT Parasitic protozoan infections represent a major health burden in the developing world and contribute significantly to morbidity and mortality. These infections are often associated with considerable variability in clinical presentation. An emerging body of work suggests that the intestinal microbiota may help to explain some of these differences in disease expression. The objective of this minireview is to synthesize recent progress in this rapidly advancing field. Studies of humans and animals and *in vitro* studies of the contribution of the intestinal microbiota to infectious disease are discussed. We hope to provide an understanding of the human-protozoal pathogen-microbiome interaction and to speculate on how that might be leveraged for treatment.

KEYWORDS microbiota, parasite, host pathogen, protozoa

Unlike for major bacterial and viral pathogens, established and readily available vaccines do not exist to prevent parasitic protozoan infections. A better understanding of the factors that influence immunity to these diseases may provide a foundation to design novel public health interventions. Transmission of the enteric protozoa typically occurs through the fecal-oral route. The intestine is densely populated by commensal bacteria well situated to influence the behavior of the protozoan parasites with which they directly interact (1). The potential influence of the microbiota on parasites is not, however, limited to the intestinal protozoa. Protozoa that live in the blood or tissue of humans may also be affected by the interplay between the gut microflora and the host metabolism and immune system (1–6). The focus of this review will therefore be the impact of the human microbiota on the parasitic protozoa that infect the intestine (*Entamoeba histolytica*, *Giardia*, *Cryptosporidium*, *Blastocystis hominis*) or vagina (*Trichomonas vaginalis*) or cause systemic infections (*Plasmodium falciparum*) (7). Changes in the composition of the intestinal microbiota may increase resistance to infection at mucosal sites, as well as alter systemic immunity to these parasites (Fig. 1).

PARASITIC PROTOZOANS AND THE SCOPE OF THE PUBLIC HEALTH IMPACT

Worldwide, diarrhea is currently the second leading cause of death in children younger than 5 years of age and is associated with around 500,000 deaths per year (8–10). Although diarrhea can be caused by many pathogens, in a large proportion of cases, the causal organism is a parasitic protozoan (11). In 2010, an estimated 357 million cases of illness with at least one of three enteric protozoa, *Entamoeba*, *Cryptosporidium*, and *Giardia*, resulted in 33,900 deaths and the loss of 2.94 million disability-adjusted life years (12). In a recent study of moderate-to-severe diarrhea in African and Asian children, *Cryptosporidium* spp. were some of the top diarrhea-associated pathogens (13).

Despite the significant health burden that protozoans cause, infections can be asymptomatic. For instance, in a Bangladeshi childhood cohort, *Entamoeba histolytica*, the causative agent of amebiasis, was found to be associated with diarrhea in only 1 of
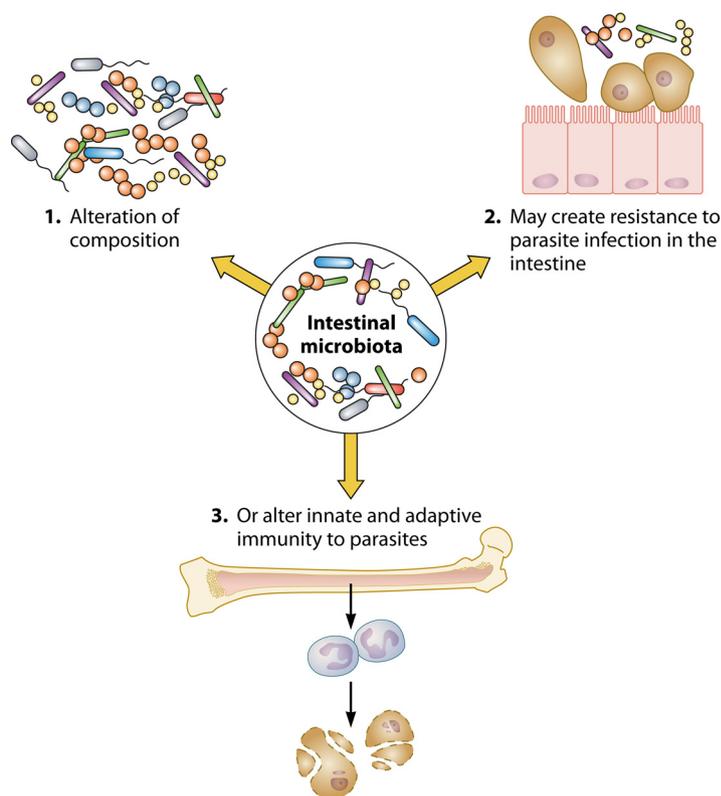
FIG 1 Host intestinal microbiota and interactions with host and parasite. Changes in the composition of the intestinal microbiota (image 1) may increase resistance to parasite infection at mucosal sites, such as the intestine, by mechanisms such as decreased virulence or parasite adherence (image 2). Changes in the microbiota may also alter systemic immunity to parasites by alteration of granulopoiesis or adaptive immunity (image 3). A better understanding of the mechanisms underlying microbiota-mediated protection may help explain clinical variability and help treat parasitic protozoan infections.

4 infections (14, 15). *Cryptosporidium* and *Giardia* infections are also marked by wide variations in clinical presentation (16–19). *Plasmodium* infections result in clinical presentations that range from asymptomatic to severe malaria and result in ~1 million deaths annually. Despite this toll, the factors that determine disease severity remain poorly understood (20). Host genetics and variation in immune response contribute to protection from parasites; however, it is increasingly clear that the intestinal microbiota may have a significant influence on the disease progression of both the enteric protozoa (1) and blood-borne malaria parasites (4).

INTESTINAL MICROBIOTA

The intestinal bacterial microbiota (21, 22) is a complex community of bacteria which is comprised of at least several hundred species. These organisms form a symbiotic relationship that influences human physiology and disease progression (23, 24). Epidemiological studies have shown that the composition of the intestinal bacterial microbiota can correlate with the development of, or resistance to, obesity (25), malnutrition (26, 27), and allergic disease (28) and may also influence cognitive function and development (29). The intestinal microbiota is not limited to prokaryotes (30), with archaea and eukaryotes potentially contributing to clinical variation (31, 32).

Microbiota compositions can vary significantly from one person to the next (33), even within healthy individuals or twins in the same household (34). Several studies have noted that the bacterial microbiota may influence the virulence of individual pathogens and potentially add variability to the outcomes of parasitic protozoan infections (1, 22). For example, coculture with *Escherichia coli* strains can augment or attenuate the virulence of *Entamoeba histolytica* (35, 36). Recently reported studies

TABLE 1 Specific components of the microbiota during human protozoan infection

Protozoan	Microbiota component	Influence	Reference
E. histolytica	Prevotellaceae	Predicted infection	38
E. histolytica	Prevotella copri	Predicted diarrhea	39
Cryptosporidium	Proteobacteria, Firmicutes, Escherichia coli CFT073, Bacillus spp., Clostridium spp.	Increased relative abundance in Cryptosporidium-negative subjects	42
G. duodenalis	Bifidobacterium	Increased relative abundance in Giardia-positive subjects	47
Blastocystis	Clostridia, Enterobacteriaceae	Increased Clostridia levels but lower Enterobacteriaceae levels in Blastocystis-positive subjects	50
T. vaginalis	Lactobacilli, Mycoplasma, Parvimonas, Sneathia	Decreased lactobacilli and increased Mycoplasma, Parvimonas, and Sneathia abundances in T. vaginalis-positive subjects	52
Plasmodium falciparum	Bifidobacterium, Streptococcus	Higher proportion of Bifidobacterium and Streptococcus organisms in a low-infection-risk group	4

highlight the impact of the microbiota on infections with enteric protozoa and on infection with extraintestinal *Plasmodium* parasites.

MUCOSAL PARASITES AND MICROBIOTA INTERACTIONS IN HUMAN POPULATIONS

Mucosal infection with the enteric protozoa *Entamoeba*, *Giardia*, *Cryptosporidium*, and *Blastocystis* can be asymptomatic or cause diarrhea, abdominal pain, and/or weight loss. The infecting parasites reside in the intestinal mucosa and therefore are surrounded by the mucosa-associated microbiota. It has been proposed that the dynamic interplay that occurs between the protozoan parasite, host microbiota, and host immune system shapes the clinical outcome of enteric infections (1, 37).

Infection with the gut parasite *Entamoeba* was significantly correlated with fecal microbiome composition and diversity. *Entamoeba* species infection was predicted by the composition of an individual's gut microbiota with 79% accuracy in a study of the farming and fishing populations in southwest Cameroon (38). One of the most important taxa in predicting an infection with *Entamoeba* was *Prevotellaceae*. In a separate independent study focused on the *E. histolytica*-associated diarrhea that is common in Bangladeshi infants, levels of *Prevotella copri*, a member of the *Prevotellaceae*, were found to be elevated in patients with diarrheagenic *E. histolytica* infections (39) (Table 1). The Cameroonian study was focused on infected adults who were not experiencing symptomatic amebiasis; therefore, it is interesting that both *P. copri* and *Prevotella stercorea* were significantly downregulated in infected individuals (38, 40, 41). Both studies suggest that microbiota composition may play a significant role during an *E. histolytica* infection. These studies also highlight the potential influence of inflammation driven by the gut microbiome in altering parasite infection outcomes (37, 39). Elevated levels of *P. copri* have been associated with severe inflammation and an increased risk of autoimmune disease and colitis, suggesting that the organism is proinflammatory (41).

Cryptosporidium, *Giardia*, *Blastocystis*, and *Trichomonas* infections may also be influenced by the gut microbiota. A retrospective study of volunteers who were originally enrolled in *Cryptosporidium* infectivity studies (42) examined the relationship between the relative abundances of several bacterial taxa commonly found in adults prior to or within 48 h of infection and infection outcomes. The patients that were protected from infection had a greater abundance of *Proteobacteria* and lower *Bacteriodetes* and *Verrucomicrobia* levels than infected subjects. There was a higher ratio of *Firmicutes* to *Bacteriodetes* in uninfected subjects than in infected subjects. Seven specific taxa had differences of at least 2.5-fold between the two groups. Specifically, uninfected subjects had increased relative abundances of the indole-producing bacteria *Escherichia coli* CFT073 and *Bacillus* spp., as well as *Clostridium* spp. In contrast, infected subjects had increased relative abundances of *Bacteroides fragilis*, *Bacteroides pyogenes*, and *Prevotella bryantii*, as well as *Akkermansia muciniphila* (Table 1). Presently, the mechanism by which increased indole production may protect from *Cryptosporidium* is unknown.

Indole may directly adversely affect the parasite or perhaps alter host tissues to enhance the innate response by increasing epithelial integrity (43) and/or stimulating anti-inflammatory pathways (42, 44).

A study of intestinal parasite infection in individuals in southern Côte d'Ivoire utilizing PCR-temporal temperature gel electrophoresis (TTGE) and quantitative PCR demonstrated that TTGE profiles clustered into four significantly different groups, i.e., groups that are positive for *Giardia duodenalis*, positive for *Entamoeba* spp. and *Blastocystis hominis*, negative for protozoa, and positive for all three parasites. Quantitative PCR of selected bacterial species in these four groups showed that there was a significant increase in the relative abundance of *Bifidobacterium* in *G. duodenalis*-positive patients. This study suggested that the tested intestinal protozoans can induce significant changes in the microbiome which result in substantially different bacterial communities (Table 1).

The relative abundances of *Faecalibacterium prausnitzii* and *E. coli* have been used as a marker of the inflammatory bowel disease (IBD)-induced dysbiosis associated with increased *E. coli* levels (45, 46). Application of this tool to samples from a patient cohort in Côte d'Ivoire suggested that the Côte d'Ivoire and Cameroonian study results were in agreement and that an increase in microbiome diversity occurs in asymptomatic *Entamoeba* species infections. The Côte d'Ivoire results also suggest that this observation may be extended and that an increase in microbiome diversity also occurred during *Blastocystis hominis* infections (47). It is controversial, however, whether *Blastocystis* can cause diarrhea (48). Part of the reason for this controversy might be due to the tremendous genetic diversity within *Blastocystis* spp. *Blastocystis hominis* consists of at least seven morphologically identical but genetically distinct organisms (49). The gut microbiome which *Blastocystis* encounters upon infecting a human host may also influence clinical outcomes. Audebert et al. compared the microbiomes of *Blastocystis*-colonized and *Blastocystis*-free patients in a case-control study design that controlled for environmental and clinical risk factors, such as seasonal variation (50). The authors also reported a higher bacterial diversity in the fecal microbiota of *Blastocystis*-colonized patients, with a higher abundance of *Clostridia* as well as a lower abundance of *Enterobacteriaceae* (Table 1). These results suggested that *Blastocystis* colonization may be associated with expansion of members of the intestinal microbiota generally associated with a healthy gut microbiota, rather than with expansion of bacteria associated with gut dysbiosis.

Trichomonas vaginalis, the causative agent of trichomoniasis and an extracellular parasite of the human urogenital tract, is the most common nonviral sexually transmitted infection globally (51). Women are disproportionally impacted by trichomoniasis, with symptomatic infection primarily impacting the vaginal mucosa. Variation in clinical presentation of disease may be impacted by the composition of the vaginal microbiota. In a study of the vaginal microbiota of *T. vaginalis*-positive and *T. vaginalis*-negative women, infection was associated with vaginal microbiota consisting of low proportions of lactobacilli (52) (Table 1). *T. vaginalis* interactions with various *Lactobacillus* species inhibit parasite interactions with human cells (53).

In summary, the referenced human studies suggest that there is a strong link between the composition of the intestinal bacterial microbiota and mucosa-associated enteric protozoa (Table 1). Future studies are needed to understand the nature of the connection and how it can be utilized for disease prevention.

PLASMODIUM AND GUT MICROBIOTA

Approximately 60% of the world's population is at risk of infection with *Plasmodium* (54, 55). However, the distribution of clinical malaria is highly heterogeneous. In studies in Kenya and Senegal, the number of clinical episodes of disease ranged from 0 to 40 per child over a 5-year period in the same community (56, 57). Clinical variation has been attributed to genetic differences. For example, heterozygous carriers of the hemoglobin variant HbS, associated with sickle cell disease, are healthy and are protected from severe forms of malaria, including cerebral malaria (58). Variation in

exposure and variance in immune response are also implicated. However, these factors may not completely explain such a large clinical variation (55, 59). The intestinal bacterial microbiota might represent an environmental factor that may contribute to this variability.

In a recent study, stool samples were collected from a cohort of Malian children and adults just before the *P. falciparum* transmission season (4). The compositions of gut bacterial communities in these individuals were determined and compared to the risks of acquiring *P. falciparum* infection and febrile malaria. A significant association was found between microbiota composition and the prospective risk of *P. falciparum* infection. The intestinal microbiota of subjects who did not become infected had a significantly higher proportion of *Bifidobacterium* and *Streptococcus* species than subjects who became infected with *P. falciparum*. However, no relationship was observed between microbiota composition and the risk of developing febrile malaria once *P. falciparum* infection was established. The authors note that this is possibly due to a lack of statistical power. The preliminary finding of an association between gut microbiota composition and *P. falciparum* infection risk suggests that alteration of the composition of the intestinal microbiota may decrease the risk of *P. falciparum* infection in areas where malaria is endemic and may potentially augment partially effective malaria vaccines (4) (Table 1).

Gut bacteria might influence extraintestinal disease via many pathways, such as by alteration of adaptive immunity and augmentation of the magnitudes of T cells and B cell-mediated responses and perhaps by enhancement of innate immune pathways via trained immunity (60). Mechanisms underlying these extraintestinal effects are poorly understood. Metabolic products, such as short-chain fatty acids (61, 62), or host-derived factors, such as damage-associated molecular pattern molecules induced by the microbiota (63, 64), might be partially responsible for these effects. The metabolite pools present in animal models with differential, microbiota-dependent susceptibility to *Plasmodium* infection varied significantly in one study, with decreases in nucleotides, amino acids, and the substrates involved in the biosynthesis of these compounds in resistant mice, along with more-robust T and B cell responses (20, 65). The gut microbiota has also been shown to have a systemic influence on serum metabolites in both animal models and humans (66, 67). Blood-stage parasites have been shown to be highly susceptible to metabolic dysregulation induced by antimalarials (68) and might also be influenced by changes induced by the microbiota. Therefore, the intestinal microbiota may influence the clinical outcome of a *Plasmodium* infection via alteration of the metabolome and modulation of innate or adaptive immunity.

ALTERATION OF THE MICROBIOTA AS A THERAPY FOR PROTOZOAN INFECTIONS?

Patient cohorts and future microbiome epidemiological studies will establish a more complete understanding of variation in clinical presentations of infection with parasitic protozoa. However, population-based studies do not allow us to test the effects of the microbiota on parasite survival and proliferation. Therefore, *in vitro* and *in vivo* disease models provide a useful tool to understand how the intestinal bacterial microbiota may influence severity and progression of infection and what mechanisms might underlie that progression.

In vitro culture models allow interactions between infecting agents and individual components of the microbiota to be analyzed. A study of the *in vitro* effects of six *Lactobacillus acidophilus* strains and *Lactobacillus johnsonii* La1 on *Giardia duodenalis* survival, for example, demonstrated that *L. johnsonii* La1 significantly inhibited the proliferation of *Giardia* trophozoites. The potential protective role of *L. johnsonii* La1 (NCC533) was independently confirmed by *in vivo* experiments with La1-treated gerbils, which were protected against *Giardia* infection and mucosal damage (69–71). In another *in vitro* study, common human commensal bacteria were cocultured with *E. histolytica*. Culture of *Lactobacillus casei* and *Enterococcus faecium* alone with amebas reduced parasite survival by 71%. When both bacteria were used in combination,

survival was reduced by 80%. A previous study demonstrated a link between decreased *Lactobacillus* and amebiasis in Indian patients (72), further supporting a potential link between these bacteria and resistance to ameba infection.

As mentioned previously, lactobacilli may impact susceptibility to *T. vaginalis* infection in women (52). Mechanisms underlying this effect are still being studied; however, inhibition of adhesion of the parasite might help explain protection. In one study, adhesion assays were carried out by incubating vaginal epithelial cells (VECs) with *T. vaginalis* and lactobacilli together and by comparing levels of parasite adhesion to nonlactobacillus recipient controls. *Lactobacillus gasseri* ATCC 9857 and CBI3 caused significant parasite adhesion inhibition in a dose-dependent manner (53).

Studies such as these may lay the foundation for utilizing individual components of the microbiota to provide cost-effective prophylactic treatment for parasite infection without the overuse of antimicrobial agents (Table 1) (73). Unfortunately, current coculture experiments do not allow us to explore the influence of the host immune system. Although differences exist between the murine and human gut microbiotas, murine models provide a powerful tool to explore host-microbiota-pathogen interactions in the context of an active immune system (74).

HOST-MICROBIOME INTERACTIONS AND MUCOSAL PARASITE INFECTION IN MURINE MODELS

The development of murine models of parasitic protozoan infections has allowed for more-detailed immunophenotyping of the mammalian host response to changes in the microbiota and its influence on infectious disease (1, 75). Murine models have also offered unexpected advancements in our understanding of interactions between the microbiota and the host due to variation in the communities of bacteria present in commercial animal vendors facilities, notably segmented filamentous bacteria (SFB) (44, 45, 93). Alteration of the intestinal microbiome in model systems and careful observation of variation between models in different environments therefore allow for a better understanding of immune factors that may help explain clinical variation in parasitic disease.

Variation in the microbiota in commercial animal facilities can result in significant changes in the progression of inflammatory and infectious diseases (76, 77). A salient example of this is colonization with a single murine commensal *Clostridium* SFB (78, 79). It was observed that C57BL/6 mice from Jackson Laboratories did not have significant interleukin 17A (IL-17A) induction in their intestinal mucosa but that C57BL/6 mice from Taconic Farms did (80). This suggested that a difference in the microbiotas of mice between these two vendors might underlie the difference in cytokine induction. Ivanov et al. (80), utilizing specific-pathogen-free mice from both vendors and germfree mice, showed that SFB, which were present in mice from Taconic Farms, were the component of the microbiota underlying the changes in immune function. Research in murine models has also shown that the immune response induced by SFB alters the severity of extraintestinal autoimmune encephalomyelitis (77, 80–82).

Recently, with a murine model of *E. histolytica* infection, we demonstrated that mice colonized with SFB are protected from experimental amebiasis (83). In exploring the responsiveness of immune cells in these mice, it was discovered that bone marrow-derived dendritic cells (BMDCs) from SFB-colonized mice produced significantly higher levels of IL-23. There was also an increase in neutrophils in the intestine, which resulted only after ameba infection (28). IL-23 is a cytokine (29) linked to induction of IL-17A and neutrophils, which in turn have been shown to be important in immunity to the ameba (30, 31). Transfer of BMDCs derived from mice colonized with SFB provided protection from *E. histolytica* infection. This work suggested that a gut-associated commensal might alter the responsiveness of bone marrow-derived cells to subsequent inflammatory challenges (Fig. 2).

In this model of amebiasis, a host damage-associated molecular pattern molecule, serum amyloid A (SAA) (84) was also increased in the sera of SFB-colonized mice compared to the level in the sera of mice lacking the commensal. Transient gut
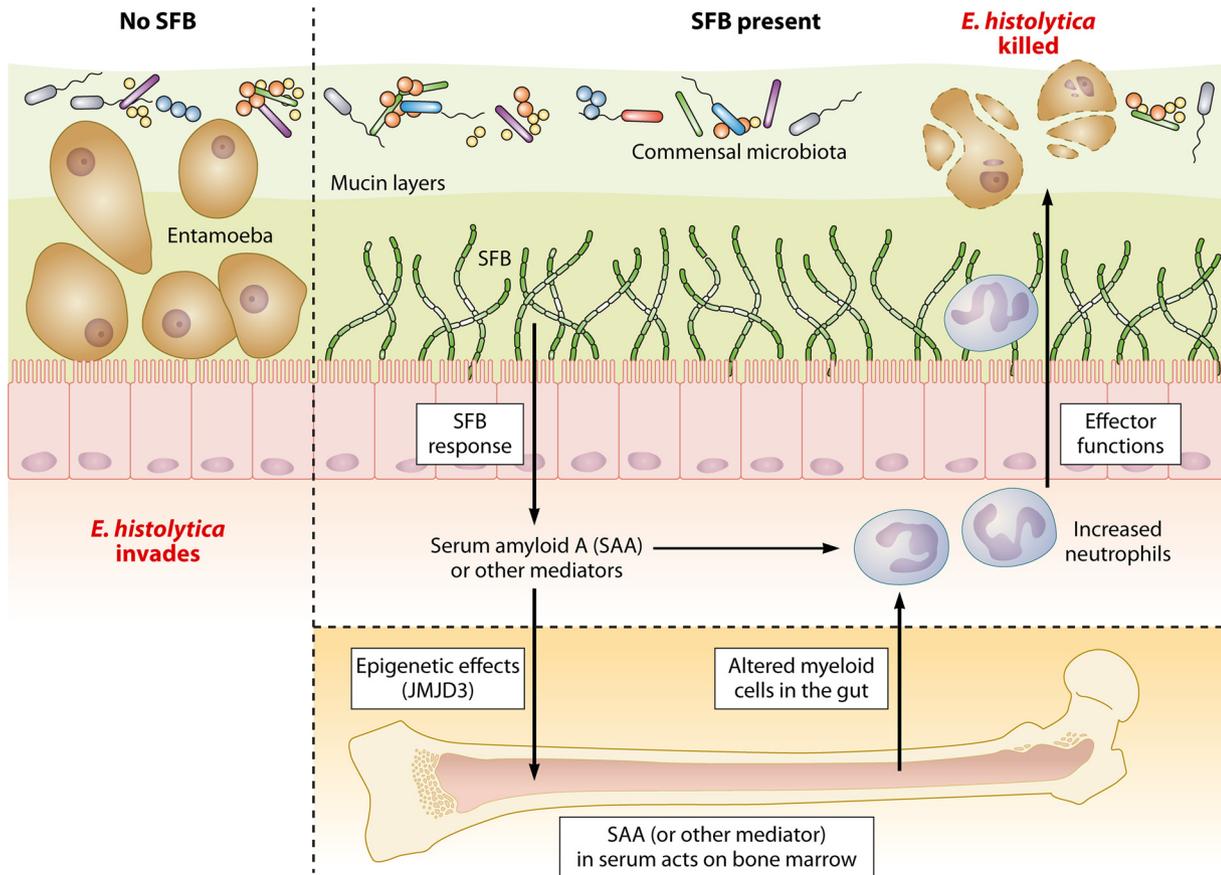
FIG 2 Model of SFB-mediated protection against *E. histolytica* colonization. SFB (segmented filamentous bacteria) colonization of the intestine may induce soluble mediators, including SAA, which may increase intestinal immune responses against ameba as well as trigger systemic epigenetic changes in bone marrow that support more-robust granulopoiesis and protection against intestinal *E. histolytica* infection. (Republished with modifications from *mBio* [83].)

colonization with SFB or SAA administration alone increased the H3K27 histone demethylase Jmjd3 in the bone marrow and persistently increased bone marrow Csf2ra expression as well as granulocyte monocyte precursors (GMPs), and protected from ameba infection. Protection was associated with increased intestinal neutrophils (63). Pharmacologic inhibition of Jmjd3 H3K27 demethylase activity during SAA treatment or blockade of granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling in SFB-colonized mice prevented GMP expansion, decreased gut neutrophils, and blocked protection from ameba infection. These results indicate that alteration of the microbiota and systemic exposure to host SAA can influence granulopoiesis and susceptibility to amebiasis, potentially via epigenetic mechanisms. Gut microbiota-marrow communication is a previously unrecognized mechanism of innate protection from ameba infection (63, 83) (Fig. 1 and 2). The intestinal microbiota likely has significant extraintestinal effects on the host immune response to parasites. These changes may be relatively long term, perhaps via induction of immune memory pathways, such as trained innate immunity (60), or via influences on adaptive immunity that are yet to be fully understood.

Antibiotic treatment which disrupts the commensal microbiota is often utilized to establish infection with pathogens in model systems. Observation of differences between the immune response in antibiotic-treated mice and untreated mice may therefore lead to insights into the role of the microbiota in the host response. In a model of *Giardia duodenalis* infection, for example, antibiotic alteration of the microbiome was shown to prevent CD8 T cell activation by *Giardia* (6). *Giardia*-infected mice that were not treated with antibiotics had more activated CD8$^+$ $\alpha\beta$ T cells in the small

intestinal lamina propria than uninfected mice. The increase in CD8$^+$ T cells was absent in antibiotic-pretreated, *Giardia*-infected mice. One potential mechanism is that during infection, the parasite promotes breakdown of the intestinal barrier. Translocation of luminal bacteria into the mucosa leads to activation of CD8$^+$ T cells; therefore, reducing the bacterial load by antibiotic treatment may reduce this and prevent pathological CD8$^+$ T cell activation (6).

Giardia duodenalis infections can have a long-term impact on human heath, and the reduction of host disaccharidases associated with *Giardia* infections may play an important role. Disaccharidases are required for the complete assimilation of nearly all carbohydrates present in food and drinks. The deficiency in disaccharidases has been thought to result from epithelial damage and shortening of the intestinal epithelial microvilli. However, in *Giardia*-infected mice, deficits in disaccharidase can be reversed by blocking CD8$^+$ T cell activation by either CD4 signaling or antibiotic treatment (6). This study suggests that differences in antibiotic usage and their effects on the human microbiome might be important factors to consider when evaluating the clinical outcome of a *Giardia* infection.

MURINE *PLASMODIUM* INFECTION AND THE MICROBIOTA

Recently, the influence of the microbiota on *Plasmodium* infection was explored by utilizing genetically similar inbred strains of mice (C57BL/6) maintained by different vendors, Jackson Laboratory, Taconic Farms, the National Cancer Institute/Charles River (NCI), and Harlan (20). C57BL/6 mice from each of these vendors were infected with *Plasmodium yoelii*. Following infection, significant differences in parasitemia were observed between the genetically identical mice from different vendors, with mice from Jackson Laboratory and Taconic Farms being resistant to the parasite. Germfree mice that received cecal transplants from "resistant" or "susceptible" mice had low and high parasite burdens, respectively, demonstrating that the intestinal microbiota may shape the severity of malaria. Resistant mice exhibited increased abundances of *Lactobacillus* and *Bifidobacterium* compared to those in susceptible mice. Additionally, susceptible mice treated with antibiotics followed by probiotics made from these bacterial genera displayed a decreased parasite burden. Consistently with differences in parasite burden, resistant mice exhibited an increased antibody profile and increased CD4$^+$ T cells and B cells compared with those of susceptible mice. Therefore, the composition of the gut microbiota may be an unidentified risk factor for severe malaria and alteration of the intestinal microbiota might augment the host response to extraintestinal parasites.

ROLE OF THE PROTOZOAN MICROBIOTA IN INFECTION AND INFLAMMATION

The primary focus of this review has been parasitic protozoa and influences of the bacterial microbiota on host immunity to these protozoa. However, it is important to note that an emerging body of work suggests that protozoa may also alter host immunity to subsequent exposures (30). Fecal-oral ingestion of *Giardia* cysts leads to varied clinical syndromes ranging from acute or chronic diarrhea to long-term asymptomatic colonization (16). A recent study of children in Bangladesh showed that early-life *Giardia* exposure neither increased nor decreased the odds of acute diarrhea from any cause. However, *Giardia* infection was a risk factor for stunting but not poor weight gain (85). It has also been noted that patients that have been infected with *Giardia* often have gut dysfunction well after their infection is cleared (86, 87). *Giardia* infection has been associated with protection from diarrhea in other cases (17, 88, 89). Mechanisms underlying these disparate outcomes in *Giardia* infection in humans are not presently well understood. However, recent work in murine models provides a demonstration of how protozoan infection might provide protection from infection while exacerbating colitis.

Tritrichomonas musculis is a common murine commensal found in wild mice and some animal colonies. It has recently been shown to cause expansion of tuft cells, a

unique epithelial cell subtype important in the generation of type 2 immune responses (90). This work suggests that commensal protozoa may be important in establishing the basic structure of the mammalian intestine. The protozoon has also just been shown to lead to expansion of adaptive Th1 cells and Th17 effector cells in the colonic mucosa. This expansion was dependent on distinct, migratory DC subsets but also required the production of IL-18 by epithelial cells. These results together with the high expression of the IL-18 receptor IL-18Rα on colonic-infiltrating effector T cells suggested that *T. musculis*-specific T cell immunity is likely initiated in the draining lymph nodes by migratory colonic DCs and is likely propagated at the tissue site by epithelial IL-18 (91). Interestingly, *T. musculis* colonization also conferred significant protection from *Salmonella* infection-driven enteritis in an IL-18-dependent manner (91). However, colonization with *T. musculis*, along with having a role as a "protistic" antibiotic, exacerbated the development of T-cell-driven colitis and resulted in the development of sporadic colorectal tumors in colonized mice. This effect of *T. musculis* was also observed in an independent study of murine colitis (92). Combined, these studies revealed novel host-protozoan interactions that led to increased mucosal host defenses while also increasing the risk of inflammatory disease.

CONCLUSIONS

Recent studies have highlighted the potential contribution of the intestinal microbiome to clinical variation in parasitic protozoan infections. The microbiome and parasites may interact in various ways, which may include (i) alteration of parasite virulence, (ii) induction of dysbiosis or perhaps even beneficial shifts in the microbiota that increase competition for the niche of the lumen of the gut, and finally, (iii) modulation of host immunity to the parasite. The courses of both mucosal and systemic parasite infection may also be shaped by specific members of the microbiota, and in turn parasite infection may alter the microbiota in such a way that the unique signature can be diagnostic of the presence of the parasite.

The exact mechanisms underlying microbiota modulation of host immunity are not yet fully understood; however, it is becoming increasingly apparent that components of the microbiota can alter both innate and adaptive immune cell populations so that a more robust response is mounted following subsequent challenge with infectious agents, including parasitic protozoa. Mechanisms underlying this shift might include the recently described concept of trained innate immunity, in which epigenetic changes enable innate immune cells to more effectively clear unrelated pathogens, and by enhancement of adaptive immunity. Ultimately, further exploration of interactions between the gut microbiome and parasitic protozoans will provide additional tools and approaches that will help in the diagnosis and treatment of infectious and inflammatory diseases. Study of protozoan interactions with the host immune system and the microbiota also help us to better understand fundamental mechanisms of mammalian immunology.

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REFERENCES

1. Bär A-K, Phukan N, Pinheiro J, Simoes-Barbosa A. 2015. The interplay of host microbiota and parasitic protozoans at mucosal interfaces: implications for the outcomes of infections and diseases. PLoS Negl Trop Dis 9:e0004176. https://doi.org/10.1371/journal.pntd.0004176.
2. Abraham NM, Liu L, Jutras BL, Yadav AK, Narasimhan S, Gopalakrishnan V, Ansari JM, Jefferson KK, Cava F, Jacobs-Wagner C, Fikrig E. 2017. Pathogen-mediated manipulation of arthropod microbiota to promote infection. Proc Natl Acad Sci U S A 114:E781–E790. https://doi.org/10.1073/pnas.1613422114.
3. Gendrin M, Rodgers FH, Yerbanga RS, Ouédraogo JB, Basáñez M-G, Cohuet A, Christophides GK. 2015. Antibiotics in ingested human blood affect the mosquito microbiota and capacity to transmit malaria. Nat Commun 6:5921. https://doi.org/10.1038/ncomms6921.
4. Yooseph S, Kirkness EF, Tran TM, Harkins DM, Jones MB, Torralba MG, O'Connell E, Nutman TB, Doumbo S, Doumbo OK, Traore B, Crompton PD, Nelson KE. 2015. Stool microbiota composition is associated with the prospective risk of Plasmodium falciparum infection. BMC Genomics 16:631. https://doi.org/10.1186/s12864-015-1819-3.
5. Buffie CG, Pamer EG. 2013. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 13:790–801. https://doi.org/10.1038/nri3535.
6. Keselman A, Li E, Maloney J, Singer SM. 2016. The microbiota contributes

to CD8⁺ T cell activation and nutrient malabsorption following intestinal infection with Giardia duodenalis. Infect Immun 84:2853–2860. https://doi.org/10.1128/IAI.00348-16.

7. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, et al. 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2095–2128. https://doi.org/10.1016/S0140-6736(12)61728-0.

8. GBD 2013 DALYs and Collaborators HALE, Murray CJ, Barber RM, Foreman KJ, Abbasoglu Ozgoren A, Abd-Allah F, Abera SF, Aboyans V, Abraham JP, Abubakar I, Abu-Raddad LJ, Abu-Rmeileh NM, Achoki T, Ackerman IN, Ademi Z, Adou AK, Adsuar JC, Afshin A, Agardh EE, Alam SS, Alasfoor D, Albittar MI, Alegretti MA, Alemu ZA, Alfonso-Cristancho R, Alhabib S, Ali R, Alla F, Allebeck P, Almazroa MA, Alsharif U, Alvarez E, Alvis-Guzman N, Amare AT, Ameh EA, Amini H, Ammar W, Anderson HR, Anderson BO, Antonio CA, Anwari P, Arnlöv J, Arsic Arsenijevic VS, Artaman A, Asghar RJ, Assadi R, Atkins LS, Avila MA, Awuah B, Bachman VF, Badawi A, Bahit MC, Balakrishnan K, et al. 2015. Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990–2013: quantifying the epidemiological transition. Lancet 386:2145–2191. https://doi.org/10.1016/S0140-6736(15)61340-X.

9. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M, Mathers C, Black RE, Child Health Epidemiology Reference Group of WHO and UNICEF. 2012. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 379:2151–2161. https://doi.org/10.1016/S0140-6736(12)60560-1.

10. GBD 2015 Mortality and Causes of Death Collaborators. 2015. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 385:117–171. https://doi.org/10.1016/S0140-6736(14)61682-2.

11. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 382:209–222. https://doi.org/10.1016/S0140-6736(13)60844-2.

12. Torgerson PR, Devleesschauwer B, Praet N, Speybroeck N, Willingham AL, Kasuga F, Rokni MB, Zhou X-N, Fèvre EM, Sripa B, Gargouri N, Fürst T, Budke CM, Carabin H, Kirk MD, Angulo FJ, Havelaar A, de Silva N. 2015. World Health Organization estimates of the global and regional disease burden of 11 foodborne parasitic diseases, 2010: a data synthesis. PLoS Med 12:e1001920. https://doi.org/10.1371/journal.pmed.1001920.

13. Liu J, Platts-Mills JA, Juma J, Kabir F, Nkeze J, Okoi C, Operario DJ, Uddin J, Ahmed S, Alonso PL, Antonio M, Becker SM, Blackwelder WC, Breiman RF, Faruque ASG, Fields B, Gratz J, Haque R, Hossain A, Hossain MJ, Jarju S, Qamar F, Iqbal NT, Kwambana B, Mandomando I, McMurry TL, Ochieng C, Ochieng JB, Ochieng M, Onyango C, Panchalingam S, Kalam A, Aziz F, Qureshi S, Ramamurthy T, Roberts JH, Saha D, Sow SO, Stroup SE, Sur D, Tamboura B, Taniuchi M, Tennant SM, Toema D, Wu Y, Zaidi A, Nataro JP, Kotloff KL, Levine MM, Houpt ER. 2016. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet 388:1291–1301. https://doi.org/10.1016/S0140-6736(16)31529-X.

14. Haque R, Ali IM, Sack RB, Farr BM, Ramakrishnan G, Petri WA. 2001. Amebiasis and mucosal IgA antibody against the Entamoeba histolytica adherence lectin in Bangladeshi children. J Infect Dis 183:1787–1793. https://doi.org/10.1086/320740.

15. Haque R, Huston CD, Hughes M, Houpt E, Petri WAJ. 2003. Amebiasis. N Engl J Med 348:1565–1573. https://doi.org/10.1056/NEJMra022710.

16. Bartelt LA, Sartor RB. 2015. Advances in understanding Giardia: determinants and mechanisms of chronic sequelae. F1000Prime Rep 7:62. https://doi.org/10.12703/P7-62.

17. Solaymani-Mohammadi S, Singer SM. 2010. Giardia duodenalis: the double-edged sword of immune responses in giardiasis. Exp Parasitol 126:292–297. https://doi.org/10.1016/j.exppara.2010.06.014.

18. Kosek M, Alcantara C, Lima AA, Guerrant RL. 2001. Cryptosporidiosis: an update. Lancet Infect Dis 1:262–269. https://doi.org/10.1016/S1473-3099(01)00121-9.

19. Chen X-M, Keithly JS, Paya CV, LaRusso NF. 2002. Cryptosporidiosis. N Engl J Med 346:1723–1731. https://doi.org/10.1056/NEJMra013170.

20. Villarino NF, LeCleir GR, Denny JE, Dearth SP, Harding CL, Sloan SS, Gribble JL, Campagna SR, Wilhelm SW, Schmidt NW. 2016. Composition of the gut microbiota modulates the severity of malaria. Proc Natl Acad Sci U S A 113:2235–2240. https://doi.org/10.1073/pnas.1504887113.

21. Moonah SN, Jiang NM, Petri WA. 2013. Host immune response to intestinal amebiasis. PLoS Pathog 9:e1003489. https://doi.org/10.1371/journal.ppat.1003489.

22. Marie C, Petri WA. 2014. Regulation of virulence of Entamoeba histolytica. Annu Rev Microbiol 68:493–520. https://doi.org/10.1146/annurev-micro-091313-103550.

23. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. 2012. Diversity, stability and resilience of the human gut microbiota. Nature 489:220–230. https://doi.org/10.1038/nature11550.

24. Sassone-Corsi M, Raffatellu M. 2015. No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens. J Immunol 194:4081–4087. https://doi.org/10.4049/jimmunol.1403169.

25. DiBaise JK, Frank DN, Mathur R. 2012. Impact of the gut microbiota on the development of obesity: current concepts. Am J Gastroenterol Suppl 1:22–27. https://doi.org/10.1038/ajgsup.2012.5.

26. Million M, Diallo A, Raoult D. 2016. Gut microbiota and malnutrition. Microb Pathog 106:127–138. https://doi.org/10.1016/j.micpath.2016.02.003.

27. Gordon JI, Dewey KG, Mills DA, Medzhitov RM. 2012. The human gut microbiota and undernutrition. Sci Transl Med 4:137ps12. https://doi.org/10.1126/scitranslmed.3004347.

28. Fujimura KE, Lynch SV. 2015. Microbiota in allergy and asthma and the emerging relationship with the gut microbiome. Cell Host Microbe 17:592–602. https://doi.org/10.1016/j.chom.2015.04.007.

29. Mayer EA, Knight R, Mazmanian SK, Cryan JF, Tillisch K. 2014. Gut microbes and the brain: paradigm shift in neuroscience. J Neurosci 34:15490–15496. https://doi.org/10.1523/JNEUROSCI.3299-14.2014.

30. Parfrey LW, Walters WA, Knight R. 2011. Microbial eukaryotes in the human microbiome: ecology, evolution, and future directions. Front Microbiol 2:153. https://doi.org/10.3389/fmicb.2011.00153.

31. Ray K. 2016. Gut microbiota: a protective protozoan in mucosal infection. Nat Rev Gastroenterol Hepatol 13:682–682. https://doi.org/10.1038/nrgastro.2016.174.

32. Lucas López R, Grande Burgos MJ, Gálvez A, Pérez Pulido R. 2017. The human gastrointestinal tract and oral microbiota in inflammatory bowel disease: a state of the science review. APMIS 125:3–10. https://doi.org/10.1111/apm.12609.

33. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, Creasy HH, Earl AM, FitzGerald MG, Fulton RS, Giglio MG, Hallsworth-Pepin K, Lobos EA, Madupu R, Magrini V, Martin JC, Mitreva M, Muzny DM, Sodergren EJ, Versalovic J, Wollam AM, Worley KC, Wortman JR, Young SK, Zeng Q, Aagaard KM, Abolude OO, Allen-Vercoe E, Alm EJ, Alvarado L, Andersen GL, Anderson S, Appelbaum E, Arachchi HM, Armitage G, Arze CA, Ayvaz T, Baker CC, Begg L, Belachew T, Bhonagiri V, Bihan M, Blaser MJ, Bloom T, Bonazzi V, Paul Brooks J, Buck GA, Buhay CJ, Busam DA, Campbell JL, et al. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486:207–214. https://doi.org/10.1038/nature11234.

34. Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, Kau AL, Rich SS, Concannon P, Mychaleckyj JC, Liu J, Houpt E, Li JV, Holmes E, Nicholson J, Knights D, Ursell LK, Knight R, Gordon JI. 2013. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 339:548–554. https://doi.org/10.1126/science.1229000.

35. Padilla-Vaca F, Ankri S, Bracha R, Koole LA, Mirelman D. 1999. Down regulation of Entamoeba histolytica virulence by monoxenic cultivation with Escherichia coli O55 is related to a decrease in expression of the light (35-kilodalton) subunit of the Gal/GalNAc lectin. Infect Immun 67:2096–2102.

36. Galván-Moroyoqui JM, Del Carmen Domínguez-Robles M, Franco E, Meza I. 2008. The interplay between Entamoeba and enteropathogenic bacteria modulates epithelial cell damage. PLoS Negl Trop Dis 2:e266. https://doi.org/10.1371/journal.pntd.0000266.

37. Burgess SL, Petri WA, Jr. 2016. The intestinal bacterial microbiome and E. histolytica infection. Curr Trop Med Rep 3:71–74. https://doi.org/10.1007/s40475-016-0083-1.

38. Morton ER, Lynch J, Froment A, Lafosse S, Heyer E, Przeworski M, Blekhman R, Ségurel L, Greenblum S, Turnbaugh P, Borenstein E, Consortium H, Petrof E, Gloor G, Vanner S, Weese S, Carter D, David L, Maurice C, Carmody R, Gootenberg D, Button J, Goodrich J, Waters J, Poole A, Sutter J, Koren O, Knights D, Lassen K, Xavier R, Spor A, Koren O, Ley R, Blekhman R, Goodrich J, Huang K, Sun Q, Bukowski R, Dominguez-Bello M, Costello E, Contreras M, Magris M, Hidalgo G, Turnbaugh P, Ley R, Hamady M, Fraser-Liggett C, Knight R, Cho I, Blaser M, et al. 2015. Variation in rural African gut microbiota is strongly correlated with colonization by Entamoeba and subsistence. PLoS Genet 11:e1005658. https://doi.org/10.1371/journal.pgen.1005658.

39. Gilchrist CA, Petri SE, Schneider BN, Reichman DJ, Jiang N, Begum S, Watanabe K, Jansen CS, Elliott KP, Burgess SL, Ma JZ, Alam M, Kabir M, Haque R, Petri WA. 2016. Role of the gut microbiota of children in diarrhea due to the protozoan parasite Entamoeba histolytica. J Infect Dis 213:1579–1585. https://doi.org/10.1093/infdis/jiv772.

40. Hofer U. 2014. Microbiome: pro-inflammatory Prevotella? Nat Rev Microbiol 12:5. https://doi.org/10.1038/nrmicro3180.

41. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, Rostron T, Cerundolo V, Pamer EG, Abramson SB, Huttenhower C, Littman DR. 2013. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. eLife 2:e01202. https://doi.org/10.7554/eLife.01202.

42. Chappell CL, Darkoh C, Shimmin L, Farhana N, Kim D-K, Okhuysen PC, Hixson J. 2016. Fecal indole as a biomarker of susceptibility to Cryptosporidium infection. Infect Immun 84:2299–2306. https://doi.org/10.1128/IAI.00336-16.

43. Shimada Y, Kinoshita M, Harada K, Mizutani M, Masahata K, Kayama H, Takeda K. 2013. Commensal bacteria-dependent indole production enhances epithelial barrier function in the colon. PLoS One 8:e80604. https://doi.org/10.1371/journal.pone.0080604.

44. Jin U-H, Lee S-O, Sridharan G, Lee K, Davidson LA, Jayaraman A, Chapkin RS, Alaniz R, Safe S. 2014. Microbiome-derived tryptophan metabolites and their aryl hydrocarbon receptor-dependent agonist and antagonist activities. Mol Pharmacol 85:777–788. https://doi.org/10.1124/mol.113.091165.

45. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux J-J, Blugeon S, Bridonneau C, Furet J-P, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottière HM, Doré J, Marteau P, Seksik P, Langella P. 2008. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 105:16731–16736. https://doi.org/10.1073/pnas.0804812105.

46. Lopez-Siles M, Martinez-Medina M, Busquets D, Sabat-Mir M, Duncan SH, Flint HJ, Aldeguer X, Garcia-Gil LJ. 2014. Mucosa-associated Faecalibacterium prausnitzii and Escherichia coli co-abundance can distinguish irritable bowel syndrome and inflammatory bowel disease phenotypes. Int J Med Microbiol 304:464–475. https://doi.org/10.1016/j.ijmm.2014.02.009.

47. Iebba V, Santangelo F, Totino V, Pantanella F, Monsia A, Di Cristanziano V, Di Cave D, Schippa S, Berrilli F, D'Alfonso R. 2016. Gut microbiota related to Giardia duodenalis, Entamoeba spp and Blastocystis hominis infections in humans from Côte d'Ivoire. J Infect Dev Ctries 10:1035–1041. https://doi.org/10.3855/jidc.8179.

48. Stenzel DJ, Boreham PF. 1996. Blastocystis hominis revisited. Clin Microbiol Rev 9:563–584.

49. Clark CG. 1997. Extensive genetic diversity in Blastocystis hominis. Mol Biochem Parasitol 87:79–83. https://doi.org/10.1016/S0166-6851(97)00046-7.

50. Audebert C, Even G, Cian A, Safadi El D, Certad G, Delhaes L, Pereira B, Nourrisson C, Poirier P, Wawrzyniak I, Delbac F, Morelle C, Bastien P, Lachaud L, Bellanger A-P, Botterel F, Candolfi E, Desoubeaux G, Morio F, Pomares C, Rabodonirina M, Loywick A, Merlin S, Viscogliosi E, Chabé M, Alaoui El H, Scanlan PD, Denoeud F, Wawrzyniak I, Wawrzyniak I, Lim MX, Wu Z, Mirza H, Tan KSW, Wu Z, Mirza H, Teo JDW, Tan KSW, Scanlan PD, Marchesi JR, Scanlan PD, Petersen AM, Krogsgaard LR, Engsbro AL, Stensvold CR, Nielsen HV, Bytzer P, Rossen NG, Manichanh C, Borruel N,

et al. 2016. Colonization with the enteric protozoa Blastocystis is associated with increased diversity of human gut bacterial microbiota. Sci Rep 6:25255. https://doi.org/10.1038/srep25255.

51. WHO. 2014. Prevalence and incidence of selected sexually transmitted infections. WHO, Geneva, Switzerland.

52. Brotman RM, Bradford LL, Conrad M, Gajer P, Ault K, Peralta L, Forney LJ, Carlton JM, Abdo Z, Ravel J. 2012. Association between Trichomonas vaginalis and vaginal bacterial community composition among reproductive-age women. Sex Transm Dis 39:807–812. https://doi.org/10.1097/OLQ.0b013e3182631c79.

53. Phukan N, Parsamand T, Brooks AES, Nguyen TNM, Simoes-Barbosa A. 2013. The adherence of Trichomonas vaginalis to host ectocervical cells is influenced by lactobacilli. Sex Transm Infect 89:455–459. https://doi.org/10.1136/sextrans-2013-051039.

54. Noor AM, Kinyoki DK, Mundia CW, Kabaria CW, Mutua JW, Alegana VA, Fall IS, Snow RW. 2014. The changing risk of Plasmodium falciparum malaria infection in Africa: 2000-10: a spatial and temporal analysis of transmission intensity. Lancet 383:1739–1747. https://doi.org/10.1016/S0140-6736(13)62566-0.

55. Ndungu FM, Marsh K, Fegan G, Wambua J, Nyangweso G, Ogada E, Mwangi T, Nyundo C, Macharia A, Uyoga S, Williams TN, Bejon P. 2015. Identifying children with excess malaria episodes after adjusting for variation in exposure: identification from a longitudinal study using statistical count models. BMC Med 13:183. https://doi.org/10.1186/s12916-015-0422-4.

56. Mwangi TW, Fegan G, Williams TN, Kinyanjui SM, Snow RW, Marsh K, Marsh K, Kinyanjui S, Bonnet S, Paul R, Gouagna C, Safeukui I, Meunier J, Mwangi T, Ross A, Snow R, Marsh K, Trape J, Rogier C, Rogier C, Trape J, Trape J, Pison G, Spiegel A, Enel C, Rogier C, Rogier C, Imbert P, Tall A, Sokhna C, Spiegel A, Rogier C, Tall A, Diagne N, Fontenille D, Spiegel A, Woolhouse M, Dye C, Etard J, Smith T, Charlwood J, Smith D, Dushoff J, Snow R, Hay S, Elissa N, Migot-Nabias F, Luty A, Renaut A, Toure F, et al. 2008. Evidence for over-dispersion in the distribution of clinical malaria episodes in children. PLoS One 3:e2196. https://doi.org/10.1371/journal.pone.0002196.

57. Rono J, Färnert A, Murungi L, Ojal J, Kamuyu G, Guleid F, Nyangweso G, Wambua J, Kitsao B, Olotu A, Marsh K, Osier FH. 2015. Multiple clinical episodes of Plasmodium falciparum malaria in a low transmission intensity setting: exposure versus immunity. BMC Med 13:114. https://doi.org/10.1186/s12916-015-0354-z.

58. Min-Oo G, Gros P. 2005. Erythrocyte variants and the nature of their malaria protective effect. Cell Microbiol 7:753–763. https://doi.org/10.1111/j.1462-5822.2005.00524.x.

59. Gong L, Parikh S, Rosenthal PJ, Greenhouse B, Williams T, Kwiatkowski D, Luoni G, Mackinnon M, Mwangi T, Snow R, Marsh K, Williams T, Taylor S, Cerami C, Fairhurst R, Piel F, Patil A, Howes R, Nyangiri O, Gething P, Williams T, Weatherall D, Hay S, Beet E, Allison A, Brain P, Ingram V, Bunn H, Haldane J, Gilles H, Fletcher K, Hendrickse R, Lindner R, Reddy S, Allan N, Hill A, Allsopp C, Kwiatkowski D, Anstey N, Twumasi P, Rowe P, Bennett S, Brewster D, McMichael A, Greenwood B, May J, Evans J, Timmann C, Ehmen C, et al. 2013. Biochemical and immunological mechanisms by which sickle cell trait protects against malaria. Malar J 12:317. https://doi.org/10.1186/1475-2875-12-317.

60. Netea MG, Joosten LAB, Latz E, Mills KHG, Natoli G, Stunnenberg HG, ONeill LAJ, Xavier RJ. 2016. Trained immunity: a program of innate immune memory in health and disease. Science 352:aaf1098. https://doi.org/10.1126/science.aaf1098.

61. Richards JL, Yap YA, McLeod KH, Mackay CR, Mariño E. 2016. Dietary metabolites and the gut microbiota: an alternative approach to control inflammatory and autoimmune diseases. Clin Transl Immunol 5:e82. https://doi.org/10.1038/cti.2016.29.

62. Brestoff JR, Artis D. 2013. Commensal bacteria at the interface of host metabolism and the immune system. Nat Immunol 14:676–684. https://doi.org/10.1038/ni.2640.

63. Burgess SL, Saleh M, Cowardin CA, Buonomo E, Noor Z, Watanabe K, Abhyankar M, Lajoie S, Wills-Karp M, Petri WA. 2016. Role of serum amyloid A, GM-CSF and bone marrow granulocyte-monocyte precursor expansion in segmented filamentous bacteria-mediated protection from Entamoeba histolytica. Infect Immun 84:e00316-16. https://doi.org/10.1128/IAI.00316-16.

64. De Palma G, Blennerhassett P, Lu J, Deng Y, Park AJ, Green W, Denou E, Silva MA, Santacruz A, Sanz Y, Surette MG, Verdu EF, Collins SM, Bercik P, Larkin W, Read J, Bradford K, Li M, Xue X, Shao S, Shao F, Wang W, O'Leary OF, Cryan JF, O'Mahony SM, Hyland NP, Dinan TG, Cryan JF,

Barreau F, Ferrier L, Fioramonti J, Bueno L, Gareau MG, Silva MA, Perdue MH, Varghese AK, Lippmann M, Bress A, Nemeroff CB, Plotsky PM, Monteggia LM, Aisa B, Chung EK, Moloney RD, Hyland NP, Barreau F, Ferrier L, Fioramonti J, Bueno L, Gareau MG, et al. 2015. Microbiota and host determinants of behavioural phenotype in maternally separated mice. Nat Commun 6:7735. https://doi.org/10.1038/ncomms8735.

65. Stough JMA, Dearth SP, Denny JE, LeCleir GR, Schmidt NW, Campagna SR, Wilhelm SW. 2016. Functional characteristics of the gut microbiome in C57BL/6 mice differentially susceptible to Plasmodium yoelii. Front Microbiol 7:1520. https://doi.org/10.3389/fmicb.2016.01520.

66. Swann JR, Want EJ, Geier FM, Spagou K, Wilson ID, Sidaway JE, Nicholson JK, Holmes E. 2011. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. Proc Natl Acad Sci U S A 108(Suppl):4523–4530. https://doi.org/10.1073/pnas.1006734107.

67. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BAH, Forslund K, Hildebrand F, Prifti E, Falony G, Le Chatelier E, Levenez F, Doré J, Mattila I, Plichta DR, Pöhö P, Hellgren LI, Arumugam M, Sunagawa S, Vieira-Silva S, Jørgensen T, Holm JB, Trošt K, MetaHIT Consortium, Kristiansen K, Brix S, Raes J, Wang J, Hansen T, Bork P, Brunak S, Oresic M, Ehrlich SD, Pedersen O. 2016. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature 535: 376–381. https://doi.org/10.1038/nature18646.

68. Cobbold SA, Chua HH, Nijagal B, Creek DJ, Ralph SA, McConville MJ. 2016. Metabolic dysregulation induced in Plasmodium falciparum by dihydroartemisinin and other front-line antimalarial drugs. J Infect Dis 213:276–286. https://doi.org/10.1093/infdis/jiv372.

69. Pérez PF, Minnaard J, Rouvet M, Knabenhans C, Brassart D, De Antoni GL, Schiffrin EJ. 2001. Inhibition of Giardia intestinalis by extracellular factors from lactobacilli: an in vitro study. Appl Environ Microbiol 67:5037–5042. https://doi.org/10.1128/AEM.67.11.5037-5042.2001.

70. Humen MA, De Antoni GL, Benyacoub J, Costas ME, Cardozo MI, Kozubsky L, Saudan K-Y, Boenzli-Bruand A, Blum S, Schiffrin EJ, Pérez PF. 2005. Lactobacillus johnsonii La1 antagonizes Giardia intestinalis in vivo. Infect Immun 73:1265–1269. https://doi.org/10.1128/IAI.73.2.1265-1269.2005.

71. Berrilli F, Di Cave D, Cavallero S, D'Amelio S. 2012. Interactions between parasites and microbial communities in the human gut. Front Cell Infect Microbiol 2:141. https://doi.org/10.3389/fcimb.2012.00141.

72. Verma AK, Verma R, Ahuja V, Paul J. 2012. Real-time analysis of gut flora in Entamoeba histolytica infected patients of Northern India. BMC Microbiol 12:183. https://doi.org/10.1186/1471-2180-12-183.

73. Sarjapuram N, Mekala N, Singh M, Tatu U. 2017. The potential of Lactobacillus casei and Enterococcus faecium combination as a preventive probiotic against Entamoeba. Probiotics Antimicrob Proteins 9:142–149. https://doi.org/10.1007/s12602-016-9232-z.

74. Nguyen TLA, Vieira-Silva S, Liston A, Raes J. 2015. How informative is the mouse for human gut microbiota research? Dis Model Mech 8:1–16. https://doi.org/10.1242/dmm.017400.

75. Houpt ER, Glembocki DJ, Obrig TG, Moskaluk CA, Lockhart LA, Wright RL, Seaner RM, Keepers TR, Wilkins TD, Petri WA. 2002. The mouse model of amebic colitis reveals mouse strain susceptibility to infection and exacerbation of disease by CD4+ T cells. J Immunol 169:4496–4503. https://doi.org/10.4049/jimmunol.169.8.4496.

76. Heczko U, Abe A, Finlay BB. 2000. Segmented filamentous bacteria prevent colonization of enteropathogenic Escherichia coli O103 in rabbits. J Infect Dis 181:1027–1033. https://doi.org/10.1086/315348.

77. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. 2011. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 108(Suppl):4615–4622. https://doi.org/10.1073/pnas.1000082107.

78. Chase DG, Erlandsen SL. 1976. Evidence for a complex life cycle and endospore formation in the attached, filamentous, segmented bacterium from murine ileum. J Bacteriol 127:572–583.

79. Kuwahara T, Ogura Y, Oshima K, Kurokawa K, Ooka T, Hirakawa H, Itoh T, Nakayama-Imaohji H, Ichimura M, Itoh K, Ishifune C, Maekawa Y, Yasutomo K, Hattori M, Hayashi T. 2011. The lifestyle of the segmented filamentous bacterium: a non-culturable gut-associated immunostimulating microbe inferred by whole-genome sequencing. DNA Res 18: 291–303. https://doi.org/10.1093/dnares/dsr022.

80. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139:485–498. https://doi.org/10.1016/j.cell.2009.09.033.

81. Caselli M, Holton J, Boldrini P, Vaira D, Calo G. 2010. Morphology of segmented filamentous bacteria and their patterns of contact with the follicle-associated epithelium of the mouse terminal ileum: implications for the relationship with the immune system. Gut Microbes 1:367–372. https://doi.org/10.4161/gmic.1.6.14390.

82. Kriegel MA, Sefik E, Hill JA, Wu HJ, Benoist C, Mathis D. 2011. Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. Proc Natl Acad Sci U S A 108: 11548–11553. https://doi.org/10.1073/pnas.1108924108.

83. Burgess SL, Buonomo E, Carey M, Cowardin C, Naylor C, Noor Z, Wills-Karp M, Petri WA. 2014. Bone marrow dendritic cells from mice with an altered microbiota provide interleukin 17A-dependent protection against Entamoeba histolytica colitis. mBio 5:e01817-14. https://doi.org/10.1128/mBio.01817-14.

84. Ye RD, Sun L. 2015. Emerging functions of serum amyloid A in inflammation. J Leukoc Biol 98:923–929. https://doi.org/10.1189/jlb.3VMR0315-080R.

85. Donowitz JR, Alam M, Kabir M, Ma JZ, Nazib F, Platts-Mills JA, Bartelt LA, Haque R, Petri WA. 2016. A prospective longitudinal cohort to investigate the effects of early life giardiasis on growth and all cause diarrhea. Clin Infect Dis 63:792–797. https://doi.org/10.1093/cid/ciw391.

86. Wensaas K-A, Langeland N, Hanevik K, Mørch K, Eide GE, Rortveit G. 2012. Irritable bowel syndrome and chronic fatigue 3 years after acute giardiasis: historic cohort study. Gut 61:214–219. https://doi.org/10.1136/gutjnl-2011-300220.

87. Hanevik K, Wensaas K-A, Rortveit G, Eide GE, Mørch K, Langeland N. 2014. Irritable bowel syndrome and chronic fatigue 6 years after Giardia infection: a controlled prospective cohort study. Clin Infect Dis 59: 1394–1400. https://doi.org/10.1093/cid/ciu629.

88. Muhsen K, Cohen D, Levine MM. 2014. Can Giardia lamblia infection lower the risk of acute diarrhea among preschool children? J Trop Pediatr 60:99–103. https://doi.org/10.1093/tropej/fmt085.

89. Veenemans J, Mank T, Ottenhof M, Baidjoe A, Mbugi EV, Demir AY, Wielders JPM, Savelkoul HFJ, Verhoef H. 2011. Protection against diarrhea associated with Giardia intestinalis is lost with multi-nutrient supplementation: a study in Tanzanian children. PLoS Negl Trop Dis 5:e1158. https://doi.org/10.1371/journal.pntd.0001158.

90. Howitt MR, Lavoie S, Michaud M, Blum AM, Tran SV, Weinstock JV, Gallini CA, Redding K, Margolskee RF, Osborne LC, Artis D, Garrett WS. 2016. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. Science 351:1329–1333. https://doi.org/10.1126/science.aaf1648.

91. Chudnovskiy A, Mortha A, Kana V, Kennard A, Ramirez JD, Rahman A, Remark R, Mogno I, Ng R, Gnjatic S, Amir ED, Solovyov A, Greenbaum B, Clemente J, Faith J, Belkaid Y, Grigg ME, Merad M. 2016. Host-protozoan interactions protect from mucosal infections through activation of the inflammasome. Cell 167:444e.14–456.e14. https://doi.org/10.1016/j.cell.2016.08.076.

92. Escalante NK, Lemire P, Cruz Tleugabulova M, Prescott D, Mortha A, Streutker CJ, Girardin SE, Philpott DJ, Mallevaey T. 2016. The common mouse protozoa Tritrichomonas muris alters mucosal T cell homeostasis and colitis susceptibility. J Exp Med 213:2841–2850. https://doi.org/10.1084/jem.20161776.

93. Hufeldt MR, Nielsen DS, Vogensen FK, Midtvedt T, Hansen AK. 2010. Variation in the gut microbiota of laboratory mice is related to both genetic and environmental factors. Comp Med 60:336–347.