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Helminths and the microbiota: parts of the hygiene hypothesis

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Abstract

In modern societies, diseases that are driven by dysregulated immune responses are increasing at an alarming pace, such as inflammatory bowel diseases and diabetes. There is an urgent need to understand these epidemiological trends, which are likely to be driven by the changing environment of the last few decades. There are complex interactions between human genetic factors and this changing environment that is leading to the increasing prevalence of metabolic and inflammatory diseases. Alterations to human gut bacterial communities (the microbiota) and lowered prevalence of helminth infections are potential environmental factors contributing to immune dysregulation. Helminths have co-evolved with the gut microbiota and their mammalian hosts. This three-way interaction is beginning to be characterized and the knowledge gained may enable the design of new therapeutic strategies to treat metabolic and inflammatory diseases. However, these complex interactions need to be carefully investigated in the context of host genetic backgrounds in order to identify optimal treatment strategies. The complex nature of these interactions raises the possibility that only with highly personalized treatment, with knowledge of individual genetic and microbiota communities, will therapeutic interventions be successful for a majority of the individuals suffering from these complex diseases of immune dysregulation.

Introduction

Whilst our previous generations were faced with constant threats of diseases caused by infectious agents (accounting for substantial proportions of human mortality (1)), the current generation is facing an epidemic of diseases associated with dysregulated inflammation (2). These include many metabolic diseases (3). Through defining the mechanisms underlying obesity, diabetes and atherosclerosis (4), we now know that dysregulated or unresolved inflammation influences many of these diseases. There is no doubt from the epidemiological perspective that there is an increase in inflammatory mediated diseases over the last 50 years. Since this is a timeframe that is unfeasible for genetic changes to have occurred in the human population, environmental factors are clearly responsible for these alterations in disease landscapes. However, genetic factors are as important in determining the consequence of these changes in response to environmental changes, as they are to response

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to infections with infectious pathogens (5). Hence, it is necessary to understand the interaction between genetic and environmental factors (6) to unravel causes behind the increasing rates of inflammatory diseases.

In order to avoid expulsion, helminths have evolved mechanisms to regulate the immune system of their hosts(7). They do not benefit from replicating within the host or utilizing intra-host population genetic approaches towards avoiding the immune response, mainly through antigenic variation (a common strategy for viruses, bacteria and protozoan parasites). Therefore, helminths have evolved production of various immune regulatory molecules (8) during the process of co-evolution, which are encoded into their genomes (9). These genomes are therefore a source of immune regulatory molecules (10). The ability of helminths to regulate the mammalian immune system is a fascinating topic, which has drawn increasing interest as the potential to exploit this knowledge for the treatment of autoimmune diseases is increasingly being considered (11, 12).

Concurrently, there has been an explosion of knowledge and interest regarding the many physiological effects of commensal bacteria (often termed the microbiota) that live with their mammalian hosts(13). While they clearly affect the ability of the host to metabolize and absorb nutrients (as was previously recognized), there has been a surge of interests in how these commensal communities regulate the immune system of their hosts(14–16). At the same time, there has been an increasing appreciation of how shifts in these bacterial communities may alter the immune response of their hosts and lead to increased inflammatory responses(17). Since alterations to the microbiota clearly has immune consequences and there are dramatic differences in the microbial communities of residents in developing and developed countries (18) with different rates of inflammatory diseases, there is also much interest to relate microbial changes to the hygiene hypothesis(19).

The most widespread helminth infections in man, as well as in most other mammals, are the intestinal helminths (20–22). This is also the locale of the majority of commensal bacteria in mammals. Undoubtedly, there must be some important interactions between these organisms that reside in the same niche (23, 24). In this review, we will discuss some of the recent developments in characterizing the interactions between helminths and the microbiota and the consequences that this may have on the immune response of the mammalian hosts.

Helminth immune responses and the hygiene hypothesis

Helminth infections are generally associated with a Type 2 response, as well as with activation of an immune regulatory network(25). It is widely regarded that the Type 2 response has likely evolved to minimize the virulence of helminth infection to their mammalian hosts (26). This response enables parasite resistance (or expulsion) by limiting the number helminths that can live in our intestinal tract (27, 28) and also promoting the repair of tissue damage that is caused by the helminths that have colonized our tissues (26, 29). This response is characterized by the production of cytokines such as interleukin-4 (IL-4), IL-5, IL-9 and IL-13, which in turn activates a broad range of downstream effector mechanisms (30, 31) that are necessary for host expulsion and tolerance of helminth infections.

IL-4 and IL-13 are key components of this response and they signal through the IL-4Ra and STAT6 (32). Acting on intestinal epithelial cells (IECs), these cytokines promote goblet cell differentiation, increased mucus production, proliferation and turnover of the IECs (27, 28). In the context of the gut microbiota, these alterations to the intestinal environment may be important for maintaining the mucosal barrier and limiting aberrant inflammatory responses triggered by the luminal or adherent bacteria in the mucosa (33). When this Type 2 response is knocked out during helminth infections in mice it can lead to lethal sepsis from compromised gut integrity and leakage of gut bacteria (26). Contraction of intestinal muscles and increased fluid flow into the lumen may help flush the worms out of the gut (27, 28), but are also likely to alter the environmental niche for gut microbial communities (23).

It was first noted from field studies that the peripheral T cells from chronically helminth-infected patients are unresponsive to stimulation with parasite antigens and responses to other antigens are also reduced (7, 34). Subsequently, investigators have worked to define the regulatory mechanisms that may be reducing responses during helminth infection. In addition to the T_H2 response described above, regulatory T cells (35), regulatory B cells (36, 37) and alternatively activated (or M2) macrophages (38–40) have all been identified as important components of the immune regulatory network activated by helminth infections (31). These same mechanisms may be responsible for preventing immune dysregulation as part of the hygiene hypothesis(12).

Some of the early field evidence to relate allergies and helminths came from Gabon, where there is a high prevalence of schistosomiasis (41). Infected children had lower responses to allergen challenge (skin test to house dust mite antigen) than uninfected children (42). Importantly, deworming the children with anti-helminthics increased reactivity to allergens (43), providing evidence for a causal effect of the worms on downregulating reactivity to the skin allergens. Since this study, there have been other studies in Brazil (44) and Equador (45) that have provided supporting evidence that helminth infections could suppress allergic inflammation (46). Since allergic inflammation and helminth infections result in strong Type 2 responses, why would helminths suppress allergen reactivity? This would not be consistent with the paradigm of a T_H2 response inhibiting T_H1 or T_H17 responses, in which case we would expect helminths to suppress T_H1/T_H17 driven diseases such as type 1 diabetes(47), multiple sclerosis (48) and Crohn's disease(12). The answer may lie in the increased levels of IL-10, a highly potent immunosuppressive cytokine. IL-10 may attenuate the ability of basophils to respond to antibody-mediated activation. While the early studies were on schistosomiasis, there is also evidence that gastrointestinal nematodes can also reduce allergic inflammation in the skin (46). However, this effect did not appear to extend to a suppressive effect on developing clinical asthma, indicating perhaps that once chronic pathogenic mechanisms have been established they cannot be overturned by helminths.

The effects of helminth infection may be especially important in early life or even in pregnant mothers. A recent study in Africa enrolled 2507 pregnant women in a randomized, double blind, placebo controlled trial with different anti-helminthic drug treatment(s) to examine immune responses against infant vaccines (49) and the health outcome of offspring. There was neither a beneficial or detrimental effect on infectious disease outcomes or on

responses to Bacillus Calmette–Guérin vaccine (BCG) immunization (49, 50). Surprisingly, the incidence of eczema increased with anti-helminthic treatment (50, 51). These results suggest that deworming during pregnancy might promote allergic disease in offspring. This large randomized study provides clinical evidence that helminth infection during pregnancy may protect children from immune dysregulation, as well as indicating that helminth infection during pregnancy does not have a negative effect on the offspring.

While we have thus far described helminths in a positive light with regard to immune regulation, it is important to note though that parasitic helminths are not commensals. In fact they are perhaps the greatest of all neglected diseases (21). There is a heterogeneous immune response to helminth infections in any endemic population (31). Individuals that mount particularly weak or inappropriate immune responses to the parasites may result in carrying heavy worm burdens. These heavy worm burdens are then likely to cause pathology associated with heavy infection (20). In contrast, there will also be some individuals that mount exceedingly strong immune responses to the helminths, these individuals are likely to carry very low worm burdens, but may suffer collateral damage from immune driven pathology (29). For the majority of individuals there is a balance between tolerance (52) and resistance to the presence of the helminths, which enables a small number of parasites to survive within the host without avert pathology (31). Under these circumstances, there is an optimal interaction whereby immune reactivity and parasite-induced suppression of immune responses are balanced to minimize pathology from either heavy worm burden or immune pathology (53). Studies on Soay sheep on an isolated island in Scotland, demonstrate that there can be a balance between reproductive fitness and survival that is driven by the magnitude of an immune response (54), indicating that there is selection for tolerating the presence of helminth infections (55) with a moderate immune response, without causing collateral damage that reduces reproductive ability.

Perhaps helminths should thus be considered to be “pathobionts”, potentially disease causing organisms, which under normal circumstances live as a symbiont with their hosts (56). This also depends on how we define “normal” circumstances. Depending on the type of helminth infection, some would be more likely to be “normally” symbiotic (e.g. gastrointestinal helminths like *Trichuris* and *Ascaris*) while others would be weighed more towards the disease-causing spectrum (e.g. schistosomiasis). In general, the gastrointestinal tract is a body site more tolerant to accommodating helminth colonization without pathology.

Microbiota and the hygiene hypothesis

The populations of microbes that are found primarily in the gut of mammals are considered to be their “microbiota”. There is ever increasing evidence that the microbiota plays an important role in regulating homeostasis of their host and consequently has effects on many different disease processes (17). Many different physiological functions, including metabolism, cognitive development (57) and immune responses are affected by the composition of the microbiota. Importantly, alterations to bacterial communities in early life appear to be most important and can have long-term consequences to physiology in adult

life (58). There have been many recent reviews on the relationship between the microbiota and human diseases (59,60) and just a few examples are highlighted here.

Inflammation is an important component of many metabolic diseases (61). The relationship between obesity and the gut microbiota is among the best studied of host-microbiota interactions (62). The increase in obesity in the last few decades is striking and clearly a result of environmental alterations. While diet and lifestyle clearly are important contributors, the composition of gut microbial communities can also contribute to progression of obesity. Obesity is now widely considered to be an inflammatory disorder and disruption of specific immune pathways; especially bacterial sensors (e.g. TLR5 (63) and Inflammasome components(64)) result in phenotypes that are associated with increased weight, adiposity or other metabolic effects. Recent studies perturbing the microbiota with low sub-therapeutic doses of antibiotics during early life, has dramatic effects on adiposity in mice, especially when combined with high fat diets. These metabolic changes were also associated with alterations to immune cell function, specifically a reduction in T_H17 responses(58).

Inflammatory bowel diseases (IBD), are complex disorders that include Crohn's disease (CD) and ulcerative colitis. These autoimmune diseases are characterized by recurring inflammation in the gastrointestinal tract (65). While the causes of IBD are still to be determined, there is general consensus that abnormal responses to the gut microbiota are a key component of the disease manifestation (65, 66). Hence, more is known about the role of the microbiota in this disease, which may serve as a model for studies to better understand host-microbiota interactions (59). Imbalances in commensal bacterial populations, referred to as dysbiosis, is a common feature among IBD patients (67). Genetic risk factors are also involved in host-microbe interactions, suggesting a disease model in which certain commensal microbes drive an exaggerated inflammatory response in genetically susceptible individuals. Hence, IBDs are complex diseases and different combinations of genetic and environmental factors (e.g. the microbiota) may require different treatment options. However, animal models that recreate this multi-hit pathogenesis mechanism have been lacking, making it difficult to understand the relationship between genetic, microbial, and other environmental risk factors. We recently developed a mouse model to understand dysbiosis, in which the combination of genetic susceptibility and microbial imbalances generates intestinal inflammation (68). These types of model may enable the gene-microbe-helminth interactions in the future.

Whilst the gut microbiota is restricted to the intestinal tract, metabolites produced by the bacterial communities can influence peripheral tissues (69, 70). In a remarkable study, supplementation of dietary fiber to mice led to increased concentrations of circulating short chain fatty acids (SCFA), as a result of metabolizing the fibers by the gut microbiota(71). This increase in SCFAs in turn protected the mice from allergic inflammation(71). SCFAs have also been shown to regulate intestinal inflammation and promote differentiation of Foxp3+ Tregs(72) (69). These finding provide a potential mechanistic link between alterations to our modern diet (low fiber high fat) and increased susceptibility to inflammatory diseases. Further studies, especially translational studies in human subjects with dietary alterations (73, 74) should validate some of these hypotheses.

As a result of the Human Microbiome Project, we now have a detailed catalog of the healthy human microbiota from individuals of the developed world (75, 76). The microbiome refers to the genomes of the microbiota. However, it is clear from the few studies conducted so far that the gut microbiota of developing countries are extremely different to residents from the developed world (18, 77, 78). Notably, helminth infection status of individuals analyzed from developing countries has not been well described in most of these studies. Diet certainly would contribute significantly to differences between the developing and developed countries. But there is growing evidence that helminth infections may also have a substantial impact on the human microbiota. Recent studies have also emerged indicating that specific taxa within the microbiota of individuals living in developing countries have profound effects on the health outcome of these people; for example, promoting the recovery from cholera (79), and affecting malnutrition (80, 81). The effects of helminth infections in these interactions remain to be determined.

Helminths and microbial communities

Just as the immune system has co-evolved with the gut microbiota, it has co-evolved to tolerate the presence of helminth infections. Hence, it is undoubtedly the case that the helminths and the gut microbiota must have co-evolved also. While this is still a new area of investigation, there is growing evidence that intestinal helminths can alter the composition of the gut microbiota.

Some of the earlier experiments were conducted on pigs. *Trichuris suis* is associated with exacerbation of campylobacteriosis (82). More recent data with next generation sequencing showed that *Mucispirillum* bacteria, which colonize mucus, were substantially increased in infected animals (83). Shotgun sequencing indicated that *T. suis* infection reduces carbohydrate metabolism, coinciding with reductions in *Ruminococcus* bacterium that are cellulolytic (83). A study to compare relationship between the mucosal immune response and worm burden was possible because at a later time point (53 days) some pigs were cleared of adult worms, whereas others were still colonized, enabling the comparison of infected pigs that retained worms with pigs that had cleared worms (84). Interestingly, *Campylobacter* was more common in worm bearing pigs. Greater worm burden was also associated with increased expression of inflammatory genes, *arg1*, *cxcr2*, *c3ar1*, *il6*, *muc5ac* and *ptgs2* (84). However, the functional relationship between the inflammatory response, worm burden and bacterial communities is difficult to determine in pigs and easier to investigate in mice.

Heligmosomoides polygyrus can alter gut microbiota of healthy mice (85–87) in the large intestine, despite the parasite occupying the small intestine. Members of the *Lactobacillaceae* family were increased in abundance in several independent studies (85–87), as were the *Enterobacteria* (86, 87). Very early experiments with germ-free mice had found that fewer adult worms were recovered from these mice, which was associated with increased eosinophilia, granulomas and thickening of the small intestinal wall (88), however this finding still awaits validation in the modern germ-free setting.

Intriguingly, the abundance of *Lactobacillus* was found to correlate positively with infection intensity of *H. polygyrus*, in addition to *H. polygyrus* increasing the abundance of *Lactobacillus* (87). Indeed, the introduction of exogenous *Lactobacillus* to mice promoted establishment of the worm infection (87). This synergistic relationship between worm and bacteria was reminiscent of another nematode, *Trichuris muris*, which utilizes the cecal microbiota to provide environmental cues for hatching of the larvae (89). It is still unclear if *T. muris* can also alter the intestinal microbiota to promote expansion of bacterial taxa that can enable hatching. Since these worms need to find mates in order to produce eggs, it would be sensible to adopt such strategies. The relationship between *H. polygyrus* and *Lactobacillus* could be driven by an expansion of regulatory T cells, which is correlated with exogenous delivery of *Lactobacillus* (87) and has been demonstrated to promote *H. polygyrus* colonization. Increase in *Lactobacillaceae* is not restricted to the gastrointestinal nematodes and interestingly the liver fluke *Opisthorchis viverrini* also increases this group of organisms (90), along with the *Lachnospiraceae* and *Ruminococcaceae*. One possibility is that these alterations are driven by the Type 2 response, a common feature of these different helminth infections.

There are now a few reported studies from humans in endemic regions infected with helminths, with many others in the pipeline (unpublished). We performed a cross-sectional study on 51 individuals from two Orang Asli villages in Malaysia, of which 36 (70.6%) were infected by helminths (91). The Orang Asli are an indigenous population who live in the rural and semi-urban areas of Peninsular Malaysia where helminth infection remains prevalent. We found that the helminth-colonized individuals among these villagers had greater species richness and number of observed OTUs, compared to the uninfected individuals. Recently, we have followed up these studies with a longitudinal deworming study and confirmed that deworming decreases microbial diversity, indicating a causal relationship between helminth infections and microbial diversity (unpublished). However, a different study conducted in Ecuador did not find significant effects of helminth infection on microbial diversity(92). In this study, the investigators compared uninfected with infected children, but also compared children before and after treatment with deworming medication. There were also no significant differences in bacterial composition. Hence, there may be geographic and cultural differences that could influence these studies, or technical differences in sample storage, extraction and sequencing platforms may also affect the results.

In summary, experimental models suggest that helminths and the microbiota may interact in positive and negative feedback loops, whereby a particular worm (e.g. *H. polygyrus*) is better suited to an environment with certain bacterial communities (e.g. abundant *Lactobacilli*) and therefore produces factors that promotes the growth of bacterial communities that favors it's presence. This may also be a strategy to exclude other worms, or enteric organisms from occupying this niche. The heterogeneity of worm burdens that is typically observed in an endemically infected human population, with 15% of the population often carrying 80% of the worm burden (21, 93), may well be a reflection of this interaction. This is a testable hypothesis for future field studies. The results from endemic human studies are still variable at the moment and definitive conclusions will await further reports.

Helminths as treatment for inflammatory diseases

Gastroenterologists at the University of Iowa led the way in taking the emerging concepts of immune regulation by helminths into translational research on inflammatory bowel disease patients (94–96). *Trichuris suis* was utilized as a therapeutic agent because it produces a self-limited colonization in humans and remains isolated to the gastrointestinal tract (97). An exploratory open-label study of seven patients with IBD was first reported in 2003. In this study and subsequent clinical trials of *Trichuris suis* ova (TSO) in Iowa, there were significant improvements in patient outcomes for both UC and Crohn's disease with essentially no adverse effects (94–96). In a randomized, placebo-controlled, double-blind study of 54 subjects with moderate to severe UC, after 12 weeks of treatment, 43.3% of the individuals treated with TSO had improved symptoms compared to 16.7% in the placebo group, which was statistically significant.

Subsequently, small investigator initiated trials were established to evaluate efficacy for multiple sclerosis (98, 99), based on observations in Argentina indicating positive effects of helminth infections on MS patients (100). There was also a larger randomized double blind, placebo-controlled trial of 100 subjects with allergic rhinitis (101). The allergic rhinitis trial did not show any significant effect on symptom scores or allergic reactivity. While there was no effect on symptoms, this trial provided an indication of potential side effects for relatively healthy individuals, not suffering from chronic gastrointestinal disorders. A few side effects were documented (102), including diarrhea, excessive flatulence, and upper abdominal pain. These events peaked 30 to 50 days after the first treatment with TSO, but were generally transient (median duration of two days) and subsided. Perhaps this is an indication of alterations in gut microbiota as a result of TSO.

The other helminth studied in clinical trials is the hookworm *N. americanus*, which has been tested on asthma and celiac disease patients. TSO is being used in clinical trials with dosages as high as 7500 eggs. For *N. americanus* there may be a narrower therapeutic window between achieving effective immune modulation and unacceptable adverse events. Doses higher than 10 larvae already correlate with increased adverse events (103). In a randomized, double-blinded trial with 32 asthma subjects, the differences between the treated and placebo groups were non-significant (104). *N. americanus* has also been tested in a randomized double blind clinical trial on 20 patients with well-controlled clinically inactive celiac disease (105). In this initial study, subjects underwent a five-day gluten challenge 12 weeks post inoculation and no significant difference was observed in pathologic grade or systemic inflammatory immune response after gluten challenge between the hookworm infected and placebo treated groups. However, a biological response was elicited to *N. americanus* in these subjects with a strong mucosal T_H2 responses as well as increased IL-22 expression accompanied by declines in IFNg and IL-17A secretion from cultured duodenal pinch biopsies (106, 107). Remarkably, with a different study design (108), with escalating gluten challenges, *N. americanus* combined with gluten microchallenge showed promise in promoting tolerance to larger challenges of subsequent gluten challenge. These results clearly illustrate the importance of devising an appropriate intervention strategy when utilizing helminths as a therapeutic agent.

Whereas in the previous section, we discussed early studies on the effects of helminth infections on the microbiota of humans living in endemic regions, there is also an emerging body of work on the microbiota from experimental treatments of individuals with helminth infections. We performed a longitudinal treatment study of juvenile macaques suffering from idiopathic chronic colitis, treated with *Trichuris trichiura* eggs we received from a self-infected individual (109). This colitis condition is a major cause of required veterinary attention in primate research centers. Four of the 5 treated macaques responded positively to infection and gained weight progressively. When we examined the composition of the mucosal microbiota from the biopsy specimens, we found that microbial diversity was significantly reduced in colitic macaques compared to healthy macaques (consistent with previous reports (110)) and diversity was partially restored post treatment. The bacterial communities post treatment was also more similar to healthy macaques. These results indicated that *Trichuris* infection might reverse dysbiosis in these macaques with colitis. In a study of healthy celiac disease patients infected with *N. americanus*, fecal samples were analyzed from 8 healthy celiac disease patients on a long-term gluten-free diet before and after infection with *N. americanus* (105–107) and analyzed by 16S sequencing. The results showed a small increase in microbial species richness, but there was not a dramatic effect on community structure, diversity or relative abundance of individual bacterial species. One possibility why the effects on the microbiota was minor could be because *N. americanus* colonizes the small intestine, rather than the colon.

The only large placebo controlled trials thus far conducted with the GMP grade helminth product, TSO, (for allergic rhinitis and Crohn's disease) have failed to demonstrate significant improvement of symptoms compared to placebo alone. While these results have been discouraging, it is important to note that our understanding of how TSO may affect a wide range of patients is poor. Currently, there are no good ways to measure correlates of biological activity. With conventional drugs (e.g. antibodies and small molecules), determination of accurate pharmacokinetics is important for dosing. For a live therapeutic organism, this information is unavailable. This scenario is more similar to a therapeutic vaccine, where investigators can still measure antibody responses or T cell response to antigens as a correlate of biological activity. In the case of TSO, hatching rates and hence parasite loads could vary significantly between individuals, and is not well understood. The composition of gut microbiota from different individuals may result in different hatching rates, since hatching of the eggs has been shown to be dependent on gut bacteria (23, 89). Hence, many challenges remain for this form of new disease treatment, as is often the case for "first in class" therapeutics.

Challenges

Immunologists tend to get confused about the diverse biological properties of different helminths. Differences in life cycles, location of larvae relative to adult forms and the migration process through different body cavities, blood vessels and lymphatic systems tend to be under appreciated. "Worms are a way to induce Type 2 responses", is a common thought process for some immunologists. While it is indeed true that Type 2 responses are a general feature of most helminth infections, and indeed features of immune regulation are also shared between most helminth infections, it is also important to note that the relative

magnitude of the Type 2 response and immune regulatory response varies greatly depending on which helminth infection is being investigated (111). Indeed, the type of response is also greatly affected by the dosage of parasites used and of course the strain of mice under investigation (28).

In the same vein as above, there is often a failure to appreciate the complexities of the many different types of autoimmune or inflammatory diseases. Indeed within the same type of disease, there are huge variations in manifestations of pathology and disease course, indicating perhaps that there are actually a number of different diseases that are being categorized together, but are in effect, many separate diseases. Whereas with cancer, there is an increasing appreciation of sub-categorizing cancer types based on molecular profiles (112–116), autoimmune and inflammatory diseases are still often being treated as a single entity, because we lack a molecular and cellular understanding of the different disease manifestations.

Thus, while there are clear examples in mouse models (11), and in individual cases (117), where a particular helminth can have dramatic effects at improving disease pathology of an inflammatory disease, there is also unlimited possibilities in pairing different helminth infections with different autoimmune and inflammatory diseases, and we are poorly placed at the moment to do so, because we do not understand the complexities of the different infections and inflammatory diseases. It is therefore dangerous to predict that any helminth infection can suppress any particular inflammatory disease, because it is just as likely to have unintended consequences of exacerbating disease (118, 119). What is likely required instead is the careful study and pairing of specific helminths (or helminth products), with specific immunological features with certain specific (perhaps even subsets?) diseases. It is unlikely that any single helminth is going to have a beneficial effect on several different inflammatory diseases.

Future directions

It is clear that gut microbial communities can exert a strong influence on the host immune response (14, 15, 120). In mouse experimental models, we can determine that these effects are definitely happening in the absence of helminth infections. While we are equally certain that helminth infections can exert strong influences on the immune response of the host (121), it has not been clearly demonstrated if these immune effects can occur in the absence of gut bacteria. Hence, it is possible that certain elements of immune regulation by helminths may be caused by alterations to the bacterial communities of the host. Germ-free mice experiments with helminth infections may clarify this in the future. In a recent study on virus-helminth co-infection (122), helminths impaired antiviral immunity, but the immune regulation was still present in germ-free mice, indicating that regulation can occur independently of the microbiota. Whether this observation is a general rule will require further investigation. Clearly, we have a lot to learn about the many different types of transkingdom interactions that can occur between the mammalian hosts, viruses, bacteria, fungi and helminths. How these interactions may impact the regulation of the immune system will be the subject of future studies.

Bacterial communities have a tendency to stabilize into several distinct clusters (sometimes referred to as enterotypes(123)). These community structures appear to be relatively resilient to perturbations (13). It has been suggested that once community structures have stabilized, considerably more “activation energy” may be required to alter these structures (59). One possibility is that helminths (or the immune response to helminths) are a driving force favoring specific bacterial communities that are more immune regulatory (24). An exciting area of further research will be to characterize the dynamic interactions between helminth infections and bacterial communities to determine whether they may promote stability and resilience of certain communities, as well as decoupling the immunological effects of the microbiota from the helminths.

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