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Macrobiota — helminths as active participants and partners of the microbiota in host intestinal homeostasis

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Important insights have recently been gained in our understanding of the intricate relationship in the intestinal milieu between the vertebrate host mucosal immune response, commensal bacteria, and helminths. Helminths are metazoan worms (macrobiota) and trigger immune responses that include potent regulatory components capable of controlling harmful inflammation, protecting barrier function and mitigating tissue damage. They can secrete a variety of products that directly affect immune regulatory function but they also have the capacity to influence the composition of microbiota, which can also then impact immune function. Conversely, changes in microbiota can affect susceptibility to helminth infection, indicating that crosstalk between these two disparate groups of endobiota can play an essential role in host intestinal immune function and homeostasis.

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Intestinal commensal bacteria and helminths flourish in vertebrate hosts, an outcome of a coevolutionary dynamic that has persisted for several hundred million years. Increasing evidence suggests that this three-way partnership has resulted in complex adaptations that have shaped the physiology of each of these very different organisms in health and disease. As a result, homeostasis in vertebrates may now require the presence of both commensal microbiota and macrobiota, including helminths. The absence of either of these organisms may dispose towards a dysregulated immune system, which may favor harmful inflammatory responses that can contribute to a variety of disease states.

For both commensal bacteria and helminths, essential adaptations promote acceptance by the vertebrate host. Shared mechanisms may include immune evasion strategies, such as molecular mimicry, activation of immune regulatory pathways, or diversionary stimulation of ineffective immune responses. The evolutionary response of the vertebrate host has been to develop suites of resistance mechanisms to control and eradicate the invading organism. A fascinating strategy is where the host opts to mitigate adverse effects of infection, accommodating an organism but minimizing pathology (a non-immunological form of tolerance [1]). Such tolerance adaptations decrease the impact of the invading organism without actually reducing the burden.

Tolerance mechanisms may include both control of harmful inflammation and enhanced wound healing that together mitigate organ and tissue damage. Tolerance mechanisms may enhance fitness for the invasive organism as well as for the vertebrate host. As such, enhanced tolerance may result from combined contributions of the vertebrate host, microbiota, and macrobiota. Recent studies also suggest that commensal bacteria and helminths interact providing signals that impact their survival in the vertebrate host. Thus, there appears to be a three way multilateral partnership that supports coexistence of these quite different organisms. In this review, we will discuss recent studies elucidating how their interactions may impact health and disease.

A number of studies published over the last few years now indicate that helminth infection can alter the composition of the intestinal microbiome with respect to both species abundance and composition [2]. For example, 3 independent laboratories reported that infection with the mouse duodenal parasite *Heligmosomoides polygyrus* expanded the proportion of Lactobacillaceae and Enterobacteriaceae in the gut [3–5•]. Moreover, while chronic infection with the mouse whipworm *Trichuris muris* similarly raised Lactobacillaceae representation, it also reduced overall microbiota diversity, a factor often associated with poorer homeostatic control, reflecting an ecological imbalance in the intestinal microbial community [6,7•]. Intriguingly, removal of parasites restored the 'naïve' flora observed in uninfected mice, suggesting that helminth-induced changes in the microbiota are reversible by clearance of the macrobiota [7•]. Likewise, in wild mice (*Apodemus flavicollis*) a correlation was observed between increased

bacterial microbiota richness and helminth infections and different types of helminths elicited characteristic changes in the composition and abundance of microbiota species [8]. In humans, reports are only now emerging and involve very different sets of helminth-exposed communities (summarised in [2]); while in a Malaysian population, microbial diversity was greater in those infected with parasites [9], it is not yet clear if this is a pattern that will be found generally applicable [6].

Taken together, these studies demonstrate that parasite infection can change the composition of gut microbiota, but do not address the possibility that changes in the microbiome may also affect susceptibility of the host to parasite infection. However, two laboratories have indeed shown that introducing higher levels of Lactobacillaceae microbes can increase susceptibility to helminth infection [5•,10], raising the suggestion that helminths and certain commensal species may mutually reinforce each other's presence. The reduced susceptibility of germ-free mice to helminth infection [11] lends further support to this proposition. Recent studies further suggest that intestinal microbiota may also affect immune responses to helminths. In a murine model of schistosomiasis, antibiotic-mediated depletion of gut bacteria significantly reduced intestinal inflammation and decreased intestinal granuloma development [8,12,13].

What may be the mechanisms underlying these effects? In the case of intestinal helminths, an important factor is the physical disruption to the epithelial surface often involving a barrier breach and causing bacterial translocation. Th1/Th17 responses evoked by opportunistic bacterial exposure may dampen the Th2 mode of immunity required for parasite expulsion, as suggested by the heightened resistance of MyD88-deficient mice to intestinal helminths [14,15]. Conversely, helminths may dampen inflammatory responses to bacteria as shown in a remarkable study of idiopathic bowel disease in captive macaques who, when given *Trichuris* worm parasites, showed a shift to a counter-inflammatory Th2-dominated environment in which microbial dysregulation is reversed and barrier function is restored [16•].

Both helminths and the microbiota are frequently linked to expanded regulatory T cell (Treg) activity [17,18], and mice in which the ability of commensals to induce intestinal Tregs is compromised were found to be more resistant to *H. polygyrus* infection [19•]. More broadly, the stimulation of Treg activity has emerged as a central explanation for the beneficial effects of certain probiotic bacteria, and controlled helminth infection, in ameliorating inflammatory diseases such as allergy and autoimmune disorders [20]. An important question now arises, of whether these changes in regulation of the immune response are caused by direct effects of the helminth parasite (e.g. by, production of excretory/secretory products) or are instead an indirect effect of the altered microbiome.

Recent studies raise the possibility that the latter may in fact be an important contributor to helminth-induced immune regulation. In one report, mice were infected with *H. polygyrus*, which is known to activate regulatory T cells capable of mediating protection against allergic asthma [21] resulting in this case in increased abundance of bacteria belonging to Clostridiales. Remarkably, transfer of microbiota-rich intestinal contents from infected mice was sufficient to trigger immune regulatory populations capable of ameliorating allergic asthma in uninfected recipient mice. Further analyses showed that short chain fatty acids (SCFAs), produced by these intestinal bacteria, was essential for increased Treg cell suppressor activation and the associated production of anti-inflammatory cytokines that controlled asthma in *H. polygyrus*-infected mice [22]. Interestingly, helminths can also produce SCFAs [23], while the intestinal lumen can carry many host products such as cytokines, exosomes and even micro-RNAs [24], raising other possibilities for how each partner in the host–parasite–commensal triangle may influence the outcome of the immune response.

The extent to which helminths may, like the microbiota [25], influence the metabolic status of their host has only recently been questioned. *T. muris*-infected mice were shown to exhibit extensive changes in fecal metabolomic products [7•], although in this and other studies it remains to be determined which changes are the result of microbial compositional and biosynthetic alterations consequent upon the nematode infection.

Helminths can in any case directly modulate the vertebrate host immune response through a number of intricate mechanisms, many of which are likely to indirectly impact the microbial cohabitants. As these large multicellular parasites migrate through tissues, they cause cellular damage and release of danger signals, such as trefoil factor 2 (tff2) and adenosine, which can in turn trigger production of IL-33, IL-25 and TSLP, inducing the release of key type 2 cytokines, including IL-4 and IL-13 [26,27•]. Through a positive feedback circuit, IL-4/13 induces expansion of epithelial tuft cells, the source of IL-25, which drives further IL-13 production from both innate and adaptive lymphocytes that can mediate worm expulsion [28]. Increased IL-13 can also enhance mucous production, and a switch from Muc2 to Muc5ac that is necessary for resistance to infection [29], most probably also changing the microbial environment in the intestine.

Type 2 immunity also includes differentiation of alternatively activated (M2) macrophages and their production of factors important in tissue repair, such as RELMα [30] and insulin-like growth factor (IGF-1) [31]. Amphiregulin is also upregulated and produced by a variety of cells

including epithelial cells, innate lymphoid cells and T reg cells, and may also enhance T reg cell function [32]. As discussed above, production of type 2 cytokines and activation of Treg cells may in turn dampen type 1 inflammatory responses to intestinal bacteria by modulating both TLR signaling and the production of type 1 cytokines, including IFN-γ. Polarisation of the response in helminth infection also raises levels of the antimicrobial products angiogenin 4 [33] and RegIIIγ in the intestines of mice [34].

Taken together, the type 2 response and the M2 macrophages it induces can mitigate tissue damage associated with helminth infection and as such may substantially enhance tolerance of these eukaryotic pathogens by the vertebrate host [35]. M2 macrophages, induced through IL-4R signaling, can also mediate resistance to helminths through products such as Arginase-1 [36,37]. In this respect, the type 2 response that has evolved in the mammalian immune system can be seen to have resolved the conflict between tolerance and resistance as the same pathway mediates parasite killing as well as necessary tissue repair [38].

A further, and unexpected, feature of the type 2 response is the degree of innate memory which is established through cell phenotypes, which persist for long periods in the host thereby contributing to the memory response upon subsequent exposure [39•]. This can help explain how helminth infections may rebalance immune homeostasis by shifting the setpoint away from harmful inflammatory responses associated with type 1 immunity, and provide a new long-term context for the recent description of 'trained immunity' [40].

Helminths also have the capacity to release excretory/secretory (ES) products that can modulate immune function, a likely consequence of host:parasite coevolution. Indeed, the ability of helminth ES to recapitulate much of the suppressive impact of live parasite infection [41] is the strongest evidence that helminth immune modulation is largely a result of direct interactions with the host, although indirect effects via microbial changes will surely play a part. While ES products are primarily thought to enhance helminth fitness by downregulating protective immune responses, it is likely that they also promote tolerance mechanisms to minimize mortality of their host and insulate their own niche from inflammatory reactions. Thus, the ES of *H. polygyrus* includes a functional mimic of the most tolerogenic mammalian cytokine, TGFβ, able to induce Treg differentiation [42], as well as a separate activity which inhibits pro-inflammatory responses of dendritic cells to TLR ligand exposure [43]. Most recently, the discovery that intestinal helminth parasites release extracellular vesicles, or exosomes, loaded with both proteins and micro-RNAs, which down-regulate (in the case of *H. polygyrus*) the IL-33R [44] opens up new pathways of communication between macrobiota, commensals and the mammalian host [45].

Finally, a relatively little-explored question is whether helminths may act directly on their intestinal microbial neighbors to regulate their populations; for example, they may disrupt the bacterial niche, preferentially deplete essential nutrients, or even release anti-bacterial products. Recent work has discovered a suite of antimicrobial mechanisms which defend free-living nematodes such as *Caenorhabditis elegans* from bacterial invasion [46], so it is plausible that species evolving in the mammalian intestine have adapted these mechanisms to control the microbiome. For example, among the ES proteins secreted by the luminal-dwelling adult stage of *H. polygyrus* are at least 8 lysozymes with potential antibacterial activity [47], as well as many small polypeptides that could include defensin-like products. Future work may well, therefore, identify novel mediators for manipulating the microbiome that could promote the anti-inflammatory effects of both helminth and beneficial commensal species.

In conclusion, it should be noted that humans are thought to carry up to 1000 different bacterial species, as well as an intestinal virome that is only now being characterized [48]. In contrast, the vast majority of intestinal helminth infections of humans are accounted for by a handful of species such as *Ascaris*, hookworms, *Strongyloides*, *Taenia* and *Trichuris*. Evidently, these successful parasites have each evolved unique strategies to manipulate both the host and its microbial constituents to remarkable effect, through pathways that are only now coming to light.

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