Trends in Parasitology

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Review

Trilateral Relationship: *Ascaris*, Microbiota, and Host Cells

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Ascariasis is a globally spread intestinal nematode infection of humans and a considerable concern in pig husbandry. *Ascaris* accomplishes a complex body migration from the intestine via the liver and lung before returning to the intestine. Tissue migration and the habitat shared with a complex microbial community pose the question of how the nematode interacts with microbes and host cells from various tissues. This review addresses the current knowledge of the trilateral relationship between *Ascaris*, its microbial environment, and host cells, and discusses novel approaches targeting these interactions to combat this widespread infection of livestock and man.

Highlights

Recent studies are uncovering a complex interplay between gastrointestinal helminths, gut microbes, and the immune system.

Parasitic helminth infections are associated with alterations to the intestinal microbiome and metabolome, and these interactions are thought to influence host susceptibility to infections with nematodes and bacterial pathogens.

Technological advances make it possible to probe these interactions in considerable depth, and the large roundworm *Ascaris* presents a particularly interesting opportunity: *Ascaris* infections in pigs essentially mirror the human disease, and the pig is being rapidly developed as a human-relevant model for infectious diseases.

Relevance of *Ascaris*–Microbiota–Host Cell Interactions

Ascariasis is one of the most common human parasitic infections worldwide and a neglected tropical disease[i]. In developing countries the prevalence of soil-transmitted helminths often exceeds 10% – a large percentage of which is caused by the large roundworm *Ascaris lumbricoides* [1]. Worldwide, *Ascaris* infections cause approximately 60 000 deaths per year, mainly in children[ii], who also experience malnutrition and developmental deficits from chronic infections [2–4]. Pathogen control is complicated by several factors, such as robust eggs surviving for several years in humid soil [5], frequent reinfection despite mass treatment with anthelmintic drugs [6], an **overdispersion** (see Glossary) among hosts [7], and the lack of vaccines applicable to humans as well as pigs. Furthermore, *Ascaris* infection compromises control of other infectious agents, including *Mycobacterium tuberculosis*, *Plasmodium* spp., and HIV [8] as well as responses to non-parasite antigens, thus hampering vaccination efficacy against other pathogens [9]. Concurrently, the infestation rate of *Ascaris suum* in pig farms was estimated to reach 30–70% across Europe [10–13]. Similar to humans, adult pigs slowly develop protective immunity against *Ascaris* following recurrent exposure. However, contact with contaminated soils in organic pig farming makes it almost impossible to disrupt the infection chain. Consequently, *Ascaris* infection leads to significant economic losses due to a reduced feed conversion ratio and liver condemnations at slaughter [14]. As in humans, *A. suum* infection negatively affects the vaccination responses to other pathogens of pigs [15].

Ascaris spends most of its lifespan in the gut surrounded by microbes. Metazoans are subject to infectious threats by microbes; thus, on the one hand, microbes in the host gut may present infectious or toxic challenges for the enteric parasite *Ascaris*. For example, the pore-forming crystal protein Cry5B, derived from *Bacillus thuringiensis*, exhibits considerable anthelmintic activity, killing *Ascaris in vitro* and *in vivo* [16]. Interestingly, saprotrophic fungal strains, such as *Aspergillus fumigatus*, can inhibit larval development and viability of *A. suum* eggs [17]. Though these are soil-dwelling organisms, their nematicidal activity exemplifies direct microbial threats to *Ascaris* which may also be posed by intestinal microbes. On the other hand, microbes may also be beneficial for worms, as has been demonstrated for the free-living nematode *Caenorhabditis elegans*: microbial components may provide key nutrients [18], protect nematodes against infection

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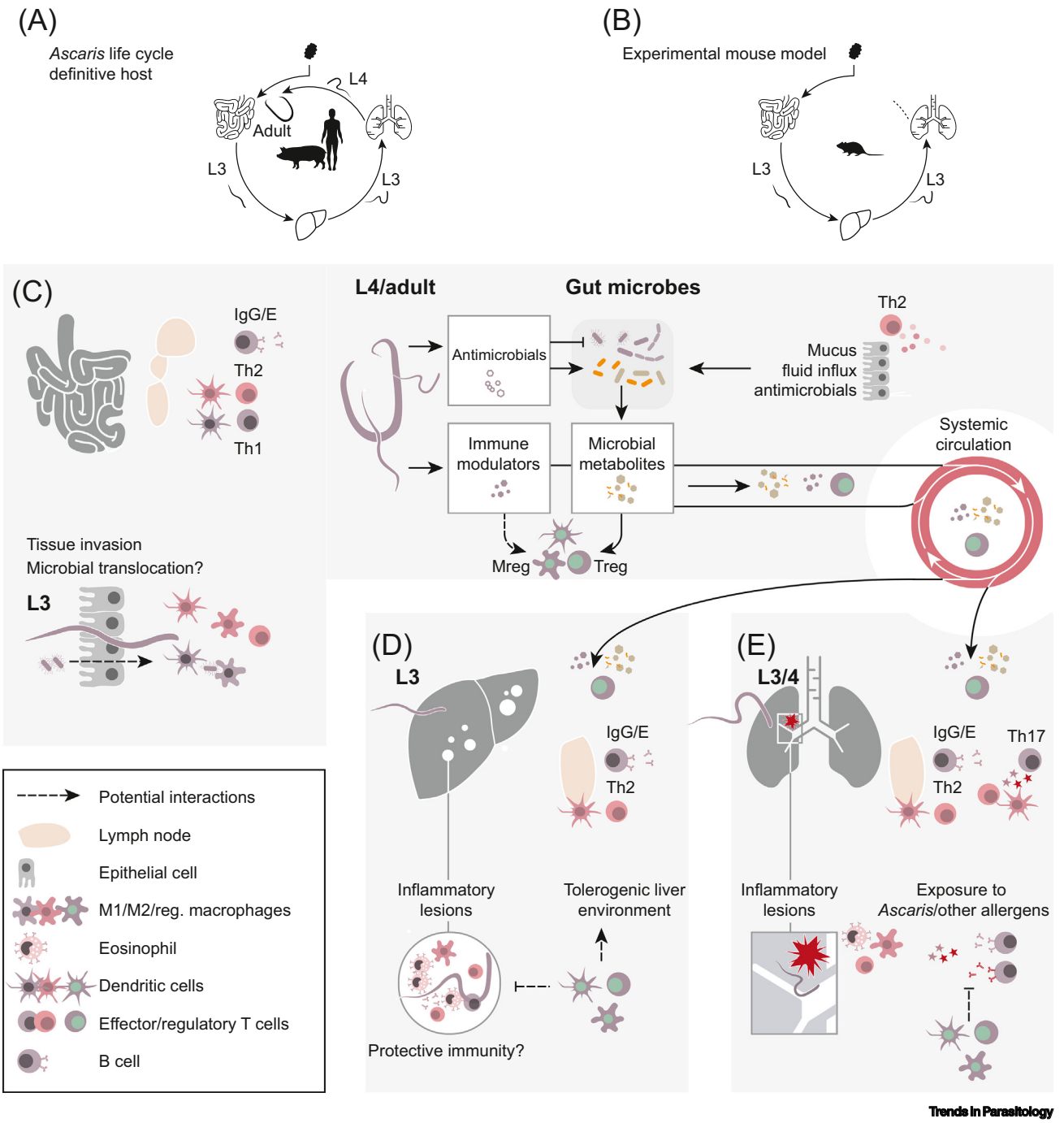
Key Figure

Potential Interactions of Parasite, Gut Microbes, and Immune Cells in *Ascaris* Infection



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[19], promote fecundity to compensate for antibacterial stress responses [20], and modulate host immune cells as shown in *Heligmosomoides polygyrus*-infected mice [21]. *Ascaris* has been shown to lead to alterations in the host **microbiome** and metabolic potential [22], in part via the release of antimicrobial factors [23]. In parallel, *Ascaris* is constantly exposed to, and attacked by, host immune and non-immune cells of different organs, depending on the localization of the life stages. It first encounters epithelial cells, followed by the cellular defense machinery of liver and lung, to finally re-encounter the cellular mucosal response of the small intestine upon completion of body migration [24]. Here we discuss the multilateral relationship (Figure 1, Key Figure) based on data from the natural hosts, as well as murine models which allow the infection to be studied up to the lung stage. This review thereby addresses the following key questions. (i) What is the role of the **microbiota** during larval invasion and body migration, as opposed to survival, maturation, and reproduction in the intestine? (ii) How does the microbial environment regulate the antiparasite immune response? (iii) What are the mechanisms and molecules exploited by *Ascaris* to modulate the microbial environment? This review focuses on interactions with intestinal organisms; readers are referred to a review by Salgame *et al.* for a discussion regarding interactions between helminths and extraintestinal pathogens [8].

First Encounter of *Ascaris* Larvae and Host Cells in a Diverse Microbial Environment

Infection begins with ingestion of eggs containing third-stage larvae which hatch within 3 h of intake. It takes the L3 larvae about 6 h to reach cecal and colonic sites where the invasion process into the mucosal tissue is initiated. The rapid mucosal penetration argues against a competition for nutrients between microbes and newly invading parasites [24]. Interestingly, for *Trichuris muris* infection in mice, signals derived from gut microbes have been shown to play a dual role. While gut microbes are needed for optimal hatching of *T. muris* larvae from ingested eggs [25], a recent study reported that structural changes of the cecal microbiome during infection suppress subsequent parasite egg hatching, protecting the host from overcrowding [26]. Another study addressing interactions between the two closely related species *T. muris* and *Trichuris suis* of mice and pigs, respectively, and a variety of Gram-positive as well as *Escherichia coli* strains demonstrated marked differences in bacterial-induced hatching between the two parasite species [27]. Hence, microbial-derived stimuli common in the definitive host, but lacking or underrepresented in off-target species, seem to contribute to host specificity. Whether a similar mechanism supports *Ascaris* infections in humans and pigs while reducing infectivity or parasite survival in other species remains to be determined.

Epithelial cells are the first host cells encountered by invading *Ascaris* larvae. Studying a microbe-free system, we have recently shown that the initial interaction of *A. suum* L3 with porcine small-

Glossary

Antimicrobial peptides (AMPs): broad-spectrum antimicrobial substances produced by organisms across the tree of life.
Excretory–secretory (E/S) products: a mixture of proteins and low-molecular-weight molecules released by parasitic worms.
Gut–lung axis: crosstalk between intestinal microbiota and lung immunity; it can be mediated by intestinal metabolites.
Loeffler syndrome: a transient inflammatory respiratory disease characterized by pulmonary eosinophilia.
Macrobiota: non-microscopic members of the intestinal biota.
Microbiome: the genetic material of the microbial communities in a particular environment.
Microbiota: the community of microbes in a particular environment.
Overdispersion: high interindividual variation in parasite burden.
Quiescent: low immune activation.
Regulatory T cells (Tregs): T cells which suppress other immune cells.
Tolerance: unresponsiveness of immune cells to substances which can usually stimulate immune responses.

Figure 1. The *Ascaris* life cycle affects three organs of both the definitive host and experimental mouse model. (A) In the definitive human and porcine host, the third larval stage hatches from ingested eggs in the small intestine and migrates to the cecum and upper colon where L3 invades the tissue. After passing through liver, lung, and airways, the fourth larval stage is swallowed and completes development into the adult stage in the small intestine. (B) The mouse model lacks the small intestinal L4 and adult stage but permits study of the liver and lung stages of the infection. (C) Immune cells are likely exposed to microbe-derived signals or translocated bacteria during the L3 invasion process, resulting in the instruction of heterogeneous T cell responses and antibody production in gut-associated lymphoid tissue. At later stages of infection, L4 and mature worms release antimicrobial factors which likely act in concert with type 2 cytokine-dependent physiological changes, resulting in structural changes of the gut microbiota. Microbial metabolites and *Ascaris*-derived immunomodulators may synergize in supporting regulatory cell populations such as regulatory T cells (Tregs) or regulatory macrophages (Mregs) and thereby repress antiparasite responses locally. Active compounds, as well as regulatory cells, may also be distributed via the circulation and act systemically. (D) In the liver, larval tissue invasion is encompassed by the development of inflammatory lesions. Experimental studies in mice lacking the intestinal L4 and adult stages suggest that hepatic protective immunity relates to the extent of liver inflammation. It is hence conceivable that circulating microbial metabolites, as well as products released by adult worms, promote tolerogenic responses and restrain protective effector/memory responses in the definitive host. (E) In the lung, considerable damage and immune cell infiltration is caused by the L4 breaching into the alveolar space. Recurrent exposure to *Ascaris* infection can result in the development of T helper (Th)2- and Th17-driven allergic responses to *Ascaris*-derived and environmental allergens. Regulatory circuits promoted by parasite products and gut-derived microbial metabolites may prevent such adverse reactions.

intestinal epithelial cells *in vitro* does not induce the typical signaling pathways associated with pathogen recognition, cell activation, and the initiation of immune responses, such as mitogen-activated protein kinase (MAPK) or NF-κB signaling [28]. This **quiescent** state of epithelial cells was also evident in the suppressed expression of immune cell attractants, as the expression of several chemokines fell below baseline levels upon larval encounter [28]. On the parasite side, dual-species RNA-sequencing (RNA-Seq) revealed the upregulation of factors potentially involved in invasion, migration, feeding, and growth of *A. suum* L3 [28]. Interestingly, proteomic analysis of **excretory–secretory** (**ES**) **products** of different *Ascaris* life stages showed that antimicrobial factors abundantly produced by the gut-dwelling L4 and adult stages are scarce in the products of the infective L3 stage commencing tissue migration [23,29]. We speculate that the infective stage quickly escapes from the potentially harmful environment posed by gut microbes and the high density of mucosal immune cells, whereas the L4 stage and adult worms permanently exposed to microbes actively modulate their microbial surrounding. Of note, it remains to be determined to what extent the quiescent state of host epithelial cells as well as the *Ascaris* gene expression and excretory profile may be altered in the presence of microbes during initial contact.

Tissue Migratory Phase

Subsequent to epithelial invasion, L3 larvae leave the intestinal tract via the portal blood stream and reach the vasculature of the liver. Migration continues via the liver sinusoids and through the hepatic parenchyma, typically in the absence of clinical signs [30]. For a better understanding of the requirements of larval migration and the immunological profile of *Ascaris* infection it is of great interest to investigate whether tissue migration leads to translocation of bacteria over breached barriers in the gut, and, shortly after, in the lung mucosa. It is conceivable that immune and endothelial cells in the gut, portal system, liver, or lung are not only exposed to parasite products, but also to bacterial components or live microbes during larval migration. While the majority of larval ascariasis cases are thought to be subclinical, without apparent bacterial-induced systemic inflammation [31], systemic spread of intestinal microbial components might pose a risk factor for sepsis as shown in various mouse models, summarized by Hübner and colleagues [32]. Furthermore, microbial pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS) or peptidoglycan, may be involved in the instruction of mixed T-helper (Th) 1/Th2 responses typically seen in the liver of *A. suum*-infected pigs and mice [33]. Of note, *A. suum*-derived molecules, including factors from pseudocoelomic fluid and the metabolites succinate and butyrate, were shown to modulate host dendritic cells (DCs) towards a regulatory phenotype [32,34]. In light of this finding, it is conceivable that *Ascaris*, like other helminths, evolved counter-regulatory mechanisms which prevent overt inflammatory responses resulting from bacterial translocation and tissue destruction during larval migration. However, the mostly subclinical course of the larval phase of infection suggests that host immune defense against bacterial infection remains largely intact.

As the liver is an organ preferentially inducing **tolerance**, the parasite might benefit from invading the liver [30]. However, larval trapping in the liver, especially during secondary *Ascaris* infection, is a common feature during hepatic migration [30]. Migrating larvae induce an inflammatory area which is visible macroscopically as white spots [31]. These are typically composed of leukocyte infiltrates dominated by eosinophils and neutrophils at early stages of formation, whereas macrophages, but also T and B cells, infiltrate the area at later stages of the response (Figure 1). These inflammatory lesions are associated with tissue repair and developing immunity to *A. suum* in pigs [31]. Still, pigs raised on contaminated pastures are continuously exposed to incoming L3, resulting in sustained liver inflammation and liver condemnation at slaughter [31].

Around 6 to 8 days postinfection, *Ascaris* reaches the lung via the blood stream. The larvae, now exceeding the diameter of the capillary bed, break into the alveolar space before being carried up the bronchial tree and coughed up to the oral cavity. Acute pulmonary ascariasis triggers considerable inflammation marked by eosinophil infiltration, a symptom complex termed **Loeffler syndrome** in human patients. Respiratory distress is evident in coughing and dyspnea. During primary infection the phase of hepato-tracheal migration is marked by the rise of innate responses including blood eosinophilia, group 2 innate lymphoid cell (ILC2) activation, Ym-1 induction in peritoneal macrophages, and eosinophil infiltration of the lung [35]. To date, surprisingly little information is available on the kinetics/phenotype of innate and adaptive responses during the tissue-migratory phase. Interestingly, a study comparing single-exposure with multiple-exposures found a significant reduction in worm burden in the lung, considerable pulmonary inflammation, and impaired pulmonary function in concert with elevated systemic Th2 and Th17 cytokines in mice repeatedly infected with *A. suum* [36]. This finding is in accordance with the demonstration of airway hyperresponsiveness and pulmonary damage provoked by *Ascaris* infection in mice [37] and the association between *Ascaris* exposure and the development of allergies/asthma demonstrated in several clinical trials with human patients [38].

In addition, studies in mice showed that *Ascaris* infection may enhance susceptibility to bacterial pathogens. Lung-stage *Ascaris*-infected mice exposed to aerosols of the lung-colonizing bacterium *Pasteurella multocida* experienced severe pneumonia and sepsis resulting in high mortality [32]. Pigs coinfected with lung-stage *Ascaris* and *E. coli* developed more severe lung pathology and bacterial translocation to lung and liver tissues [39]. Clearly, the effects of inflammatory responses to migrating *Ascaris* larvae on microbial communities of the respiratory tract and the consequences for the control of other pulmonary infections as well as respiratory health merit further investigations.

Gut–Lung Axis and Survival of *Ascaris* in the Intestine

Emerging evidence reveals an important crosstalk between the intestinal microbiota and the lungs, termed the **gut–lung axis** [40]. Changes in the composition of the gut microbiome, through either diet, antibiotic treatment, or nematode infections are linked with altered immune responses and homeostasis in the respiratory tract [40–43]. Analysis of colon contents from pigs experimentally infected with *A. suum* revealed increased total short-chain fatty acid (SCFA) concentrations with significant increases in propionate and butyrate and a trend toward increased acetate concentrations [44]. Interestingly, helminth-associated increases in SCFAs attenuated allergic airway inflammation in *H. polygyrus*-infected mice (Table 1) [44]. In an asthmatic human population, members of the gut microbiota were associated with fixed airflow obstruction and lower specific IgE response to *Ascaris* [45]. Furthermore, an experimental study in *H. polygyrus*-infected mice demonstrated that a strictly enteric helminth infection can have remote protective antiviral effects in the lung through induction of a microbiota-dependent type I interferon response [46]. Thus, the consequences of *Ascaris* migrating through the lung and dwelling in the gut impacts airway inflammatory processes through the gut–lung axis.

Ascaris infection modifies the host gut microbiota, with one study suggesting an increase in alpha diversity at 14 days post infection (dpi) [47] while another study reported a worm-burden-independent decrease in diversity indices in chronically infected pigs at 54 dpi [22]. In both instances, microbial compositional changes were more pronounced in the proximal colon compared with the feces, suggesting localized effects of *A. suum* infection on the host microbiota. Chronic infection was associated with SCFA (acetate and proprionate) production potential and levels as well as impaired microbial digestion of carbohydrates, suggesting a favorable metabolic environment for *Ascaris* with an upregulation of numerous glycosyl hydrolases, ultimately for glucose uptake

Table 1. Demonstrated and Predicted *Ascaris*–Microbiota–Host Cell Interactions

Interaction (experimental system)	Direction[a]	Outcomes	Refs
Ascaris-derived antimicrobial peptides and proteins (ASABFs, cecropins, lectins, lysozymes) (*Ascaris suum*)	A➔M A➔H	Microbial killing, microbial neutralization, immunomodulation	[23,75,76]
Ascaris-derived metabolites (e.g., SCFA, succinate) (*A. suum*)	A➔H, A➔M	Promotion of regulatory immune phenotype, altered microbiome and metabolic environment, influence bacterial motility, growth, and gene expression	[32,34,83,86–89]
Egg-hatching (*Trichuris muris, Trichuris suis*)	M➔A	Promotion or prevention of egg hatching and infection	[25–27]
Microbiota-derived anthelmintic activity/infection of nematode by microbe (*A. suum*)	M➔A	Hampered larval development, nematode killing	[16,17]
Microbiota-mediated defense of nematodes, promotion of nematode viability (*Caenorhabditis elegans, Heligmosomoides polygyrus*)	M➔A	Providing nutrients, protecting nematode against microbial infection	[18–21]
Bacterial translocation during nematode tissue migration (*A. suum*)	A➔M➔H	Increased risk of microbial infection; compromised anthelmintic immune response	[32,36,39]
Gut–lung axis (*H. polygyrus, T. suis*)	A/M➔H➔M	Nematode infection alters intestinal microbiome and metabolome, which modulates respiratory immune responses	[40–43]
Host immunomodulation by *Ascaris* (*A. suum*)	A➔H➔M	Compromised immune responses against microbes and nematode, altered microbiome and intestinal metabolome	[8,9,15]
Host immunomodulation by microbes (*H. polygyrus, Nippostrongylus brasiliensis*)	M➔H➔A	Compromised immune responses against *Ascaris*	[60–62]

[a]Interactions: A, *Ascaris*; M, microbiota; H, host cells; ➔ indicates sequence and directionality of interactions (e.g., M➔H➔A: microbiota impact host cells which then impact *Ascaris*).

[22]. Interestingly, *H. polygyrus*-infected mice also show altered intestinal microbial communities associated with increased SCFA production leading to protection against allergic asthma as a clear example of host immunomodulation via the gut–lung axis [44].

In addition, the intestine of adult ascarids contains bacteria [48,49], suggesting that *Ascaris* harbors its own intestinal microbial community. Notably, the studies published to date used culture-based methods, and the *A. suum* microbiota and **macrobiota** have not yet been characterized using modern methods. Interestingly, *A. lumbricoides*, isolated from cholera patients, was also colonized by *Vibrio cholerae* [50], suggesting that the nematode intestine could serve as a survival niche for microbial pathogens. *A. suum* can also carry non-bacterial pathogens such as the porcine epidemic diarrhea virus (PEDV) [51]. In other intestinal nematode infections, acquired host-intestinal microbes, in particular *Bacteroides thetaiotaomicron,* have been shown to promote nematode fitness and development in *T. muris* [26]. It follows that *Ascaris*, too, hosts microbes beneficial for its survival in the host intestine.

Regulation of Immune Responses to *Ascaris* by Gut Microbes

Gut microbes and intestinal worms are controlled by opposing innate and adaptive immune responses orchestrated by Th1/Th17 and Th2 cells, respectively. Humans, pigs, and mice infected with *A. lumbricoides* or *A. suum* display biased Th2 responses, and the magnitude of Th2 and associated IgE responses in infected human patients is predictive of resistance against challenge infections [52–56]. Th2 responses are optimally induced in the absence of signals favoring Th1 and Th17 differentiation [57–59], and coinfections with Th1-associated pathogens can support nematode survival by suppressing or even blocking the development of Th2 responses [60–62]. It is hence not unlikely that *Ascaris* may benefit from the deviation of immune responses when the human or porcine host is confronted with several pathogens simultaneously

(Box 1). Furthermore, microbial signals derived from the gut microbiota are shown in other systems to be sufficient to restrain Th2 responses during experimental enteric nematode infection, as MyD88-deficient mice insensitive to most Toll-like receptor (TLR)-mediated proinflammatory signals display stronger Th2 responses and more efficient parasite control against *H. polygyrus* and *T. muris* [63].

Both gut microbes and parasitic nematodes such as *H. polygyrus* stimulate the differentiation of **regulatory T cells** (**Tregs**) in order to escape elimination by the immune system [64,65]. Studies in *H. polygyrus*-infected mice suggest that microbial Treg activation confounds the optimal expression of protective Th2 responses and facilitates the prolonged survival of enteric nematodes [21,66,67]. We have shown that germ-free mice displayed reduced parasite fitness associated with low Treg/Th2 ratios and poor interleukin (IL)-10 production by intestinal Tregs during *H. polygyrus* infection [66]. Nematode-infected germ-free mice largely lacked Foxp3^{+}RORγt^{+} Tregs, a subset previously reported to depend on the presence of gut microbes and to regulate Th2-driven experimental gut inflammation and antinematode Th2 responses [66,67]. Interestingly, some microbial species also mitigate host damage during enteric nematode infection (Figure 2). *A. suum*-infected pigs demonstrated enhanced parasite-specific IgA, IgG1, and IgG2 responses, decreased small-intestinal eosinophilia, and restored intestinal glucose uptake when fed with the probiotic *Bifidobacterium animalis* subsp. *lactis* [68].

Members of the order Clostridiales were shown to expand in the gut of mice infected with the strictly enteric nematode *H. polygyrus*. This was associated with the elevated production of SCFA from dietary fibers, the expansion of Tregs, a rise in local IL-10 and transforming growth factor (TGF)-β production, and the control of allergen-induced Th2 cytokine responses in allergic asthma [44]. Notably, elevated SCFA levels were also detected in the gut of *A. suum*-infected pigs and in the majority of a small cohort of human volunteers infected with hookworms [44]. Thus, it will be interesting to determine if distinct microbiota and SCFA profiles can be linked to a Th2/

Box 1. Interactions between *Ascaris* and the Non-bacterial Intestinal Biota

Few studies have investigated how *Ascaris* interacts with non-bacterial intestinal pathogens and commensals, including viruses, fungi, protozoa, and other parasites. However, emerging work on the virome [90] and mycobiome [91] of the human intestinal tract provides the basis for the identification of interactions between bacteriome, non-bacterial communities, and the immune system of the host.

Parasite–Parasite Interactions

Multiple-species infections with other soil-transmitted helminths (*Trichuris trichiura*, *Ancylostoma duodenale, Necator americanus*) are frequently seen in areas where *Ascaris lumbricoides* is endemic, but demonstrate heterogenic associations in prevalence and egg deposition [92,93]. Low levels of interaction between *Ascaris* and other nematode species are also seen in experimental infections of pigs [94,95]. Epidemiological studies suggest that *A. lumbricoides* impairs immunity against *Giardia* spp. [96] and *Schistosoma mansoni* [97]. Furthermore, cumulative effects regarding malnourishment were demonstrated for coinfections with *Ascaris* and other helminth or protozoan parasites in both humans [98] and pigs [99].

Parasite–Virus Interactions

Strong evidence for a direct interaction between helminths, host immunity, and viruses is seen in murine coinfection models demonstrating impaired immunity against vaccinia virus in *Ascaris*-exposed mice [100].

Parasite–Fungus Interactions

Whilst the impact of *Ascaris* infection on the host mycobiome has not been addressed so far, mutual effects were observed *in vitro*. These include the demonstration of antifungal or anthelmintic properties of cecropins released by Ascarids and *Aspergillus* products, respectively [76,101], as well as the impaired embryonic development of *Ascaris* eggs exposed to saprotrophic soil fungi [17,102].

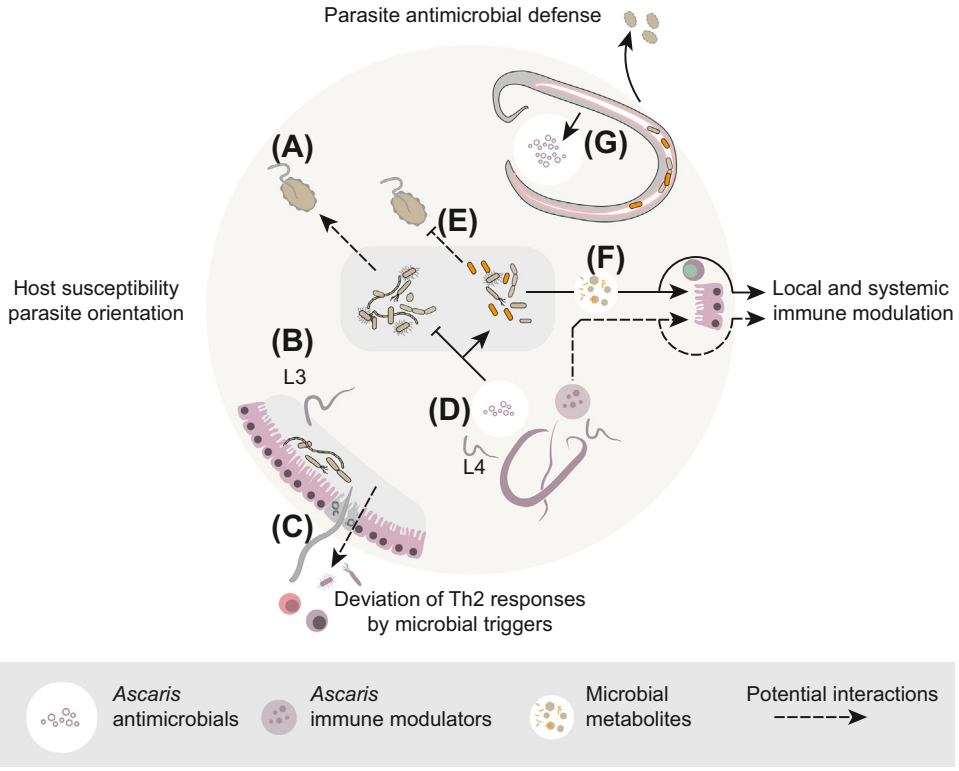

Figure 2. Potential Role of Bacteria during Invasion, Migration, and Reproduction of *Ascaris*. Upon ingestion, stimuli derived from gut microbes may affect hatching rates of *Ascaris* eggs (A), guide the L3 stage to the site of tissue invasion (B), and deviate the developing T helper (Th)2 response (C). Antimicrobial factors produced by the fourth larval and adult stages are likely involved in microbiota changes during infection (D) which may regulate larval hatching during ongoing exposure (E). Microbial metabolites and *Ascaris*-derived immunomodulators likely synergize in local and systemic immune modulation (F). The production of antimicrobials by *Ascaris* may adapt to defend against bacterial pathogens, regulate the composition of the parasite microbiome, as well as the metabolic environment, in order to secure parasite reproduction (G).

Th17 balance influencing lung migration and the effectiveness of adaptive immune responses during *Ascaris* infection.

Several studies reported the expansion of lactobacilli in mice experimentally infected with the enteric nematodes, *H. polygyrus*, *Nippostrongylus brasiliensis, Trichinella spiralis,* and *T. muris* [21,69–71]. Probiotic members of this family support the differentiation of Tregs, thereby suppressing Th2-mediated allergic airway inflammation in mice [72,73], while restricting inflammatory reactions to *Ascaris* allergens in pigs [74]. Hence, compositional changes of the microbial community, alterations in the availability of microbial danger signals, or direct contact of immune cells with gut microbes during *Ascaris* infection affects the quality of innate and adaptive immune responses against the nematode.

Mechanisms and Molecules of *Ascaris* Modulating the Microbial Environment

Nematodes also interact directly with microbes through the secretion of a variety of effector molecules (Table 1). *A. suum* has been shown to upregulate the expression of cationic **antimicrobial peptides (AMPs)** when challenged with heat-inactivated *E. coli*, namely cecropins and members of the *A. suum* antibacterial family (ASABF) [75,76]. This demonstrates that ascarids recognize and

respond to bacteria by inducing a defense response. Studies from our group have also detected ASABFs and cecropins in the ES products of intestine-dwelling L4 and adult *A. suum* [23]. These products display diverse antibacterial activities, including bacterial growth inhibition, biofilm disruption, and agglutination [23]. We have also demonstrated similar activities for the ES products of *H. polygyrus* [66]. These studies demonstrate that microbial signals are detected by nematodes, eliciting an antimicrobial response.

In addition to direct bactericidal activity, nematodes also employ nonlethal defense mechanisms. We and others have observed lectin-domain-containing proteins in *A. suum* [23], *N. brasiliensis,* and *H. polygyrus* [77]. Harcus and colleagues characterized a C-type lectin protein from *H. polygyrus* with similarities to *C. elegans* proteins previously described to be induced during bacterial infection of the worm, capable of bacterial binding and neutralization [77]. Similarly, we reported agglutinating activity of ES products from *A. suum* and *H. polygyrus* [23,66], suggesting that nonlethal defense strategies are employed by these enteric nematodes. Accordingly, *H. polygyrus* C-type lectin-domain-containing proteins are also predicted [77] to interact with host cells, and one could imagine that the proteins provide dual functions of defending the nematode against microbial and immune threats.

In addition to traditionally secreted protein effectors, intestinal parasites likely employ additional mechanisms to shape the host microbiota, including the production of extracellular vesicles (EVs) and metabolites. As with other helminths, *Ascaris* produces EVs containing nucleic acid and protein cargo [78]. While vesicular miRNAs from *H. polygyrus* demonstrate immunomodulatory functions [79], the protein components have been largely understudied. The protein components in *Ascaris* EVs contain lectins, though no other potential antimicrobial proteins were reported [78]. However, during filter-aided preparation of the EVs, small peptides are likely excluded from proteomic analysis [80]. Thus, if small AMPs are contained in EVs, this would not have been observed. While parasite–host and parasite–parasite interactions via EVs have been reported [81], to our knowledge parasite–microbiota interactions have not yet been assessed. Interestingly, bacteria also produce EVs which can enter the host circulation [82]. Whether helminths can influence the contents of bacterial EVs, and how bacterial EVs may interact with host cells or with nematodes to impact nematode fitness, remains to be determined.

Ascaris-produced metabolites may also influence the microbiota. As mentioned earlier, *A. suum* can produce SCFAs [83] which impact host immunity but also shape gut microbial communities and have implications for susceptibility to bacterial infection [84,85]. Intestinal metabolomic changes associated with the enteric nematode *H. polygyrus* promoted coinfection with *Salmonella* [86]. *In vitro* and murine models of *Salmonella* virulence show that SCFAs can impair bacterial motility and biofilm formation [87], and confer colonization resistance and limit *Salmonella* growth [88], with mixed effects on virulence gene expression [87–89]. These studies indicate that *Ascaris*-derived metabolites themselves may influence coinfection by other gut pathogens and this is worthy of *in vivo* study in pigs.

Concluding Remarks

In conclusion, *Ascaris* has developed various measures which may modulate the host microbiota by restricting or supporting the growth of individual microbial species. The larval body migration likely provides an advantage in dispersing cellular immune effector mechanisms to several sites of the host's body before the parasite settles and completes development in the gut. After settlement in the intestine the microbial diversity and composition appears to be modulated by *Ascaris*, and we speculate that this creates a metabolic environment favorable to parasite survival and reproduction. Likewise, *Ascaris* may benefit from the expansion of specific microbes which support

Outstanding Questions

Do certain gut microbes promote the establishment of *Ascaris* in the gut?

Do defensive microbes protect *Ascaris* from microbial infections?

Can the gut microbiota and the local metabolic environment be manipulated to promote anthelmintic responses or prevent *Ascaris* infection?

Does *Ascaris* alter host susceptibility to important gut pathogens, such as *Salmonella*?

Do certain bacterial species influence (i) *Ascaris* egg hatching, (ii) mucosal invasion, and (iii) tissue migration, as is seen in other helminths?

Can we identify an '*Ascaris*-microbiome', and how much interindividual variation exists in nematodes within a single host and between nematodes in different hosts?

the activity of regulatory cell populations and thereby counteract overt inflammatory reactions against the parasite. To develop novel measures of interference with recurrent *Ascaris* infection, future work will have to elucidate dependencies of *Ascaris* on specific microbial metabolites or bacterial species (see Outstanding Questions). Investigation of the microbiota hosted by *Ascaris* worms may provide a straightforward approach delineating which microbes can be considered as detrimental or beneficial to parasite fitness. In light of recent findings and technological advances, such interactions can now be investigated in far more depth, revealing novel therapeutic opportunities.

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Resources

[i]www.cdc.gov/parasites/ascariasis/index.html

[ii]www.who.int/water_sanitation_health/diseases-risks/diseases/ascariasis/en/

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