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# Intestinal immune responses to commensal and pathogenic protozoa

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The physical barrier of the intestine and associated mucosal immunity maintains a delicate homeostatic balance between the host and the external environment by regulating immune responses to commensals, as well as functioning as the first line of defense against pathogenic microorganisms. Understanding the orchestration and characteristics of the intestinal mucosal immune response during commensal or pathological conditions may provide novel insights into the mechanisms underlying microbe-induced immunological tolerance, protection, and/or pathogenesis. Over the last decade, our knowledge about the interface between the host intestinal mucosa and the gut microbiome has been dominated by studies focused on bacterial communities, helminth parasites, and intestinal viruses. In contrast, specifically how commensal and pathogenic protozoa regulate intestinal immunity is less well studied. In this review, we provide an overview of mucosal immune responses induced by intestinal protozoa, with a major focus on the role of different cell types and immune mediators triggered by commensal (*Blastocystis* spp. and *Tritrichomonas* spp.) and pathogenic (*Toxoplasma gondii*, *Giardia intestinalis*, *Cryptosporidium parvum*) protozoa. We will discuss how these various protozoa modulate innate and adaptive immune responses induced in experimental models of infection that benefit or harm the host.

## KEYWORDS

intestinal immunity, commensal, protozoa, blastocystis, *Cryptosporidium*, *Giardia*, *Toxoplasma*

## Introduction

Mucosal tissue is a physical barrier composed of biochemical and immunological components at the interface between the host and the external environment. Mucosal immunity plays a fundamental role in promoting tolerogenic immune responses in order to maintain homeostasis in addition to providing the first line of defense against pathogenic and non-pathogenic microorganisms (1).

The gastrointestinal tract is the largest mucosal tissue in the human body, which harbors a diverse community of commensals including prokaryotic bacteria, eukaryotic fungi, and protozoa, as well as other organisms such as intestinal viruses, helminth parasites, and pathogenic protozoa (Figure 1). Although commensals are commonly referred to as symbiotic microorganisms that are either non-pathogenic or beneficial (referred to as mutualists) to their host (2), it is increasingly evident that their presence plays an important role in reshaping the host immune system (3). Beyond their role in digestion and nutrient acquisition, the presence of

commensal organisms is fundamental to maintaining intestinal homeostasis and modulating the development and maturation of the immune system, which is pivotal to effectively protecting the host against pathogenic organisms (4). Thus, intestinal mucosal immunity continuously functions to maintain a delicate balance to the commensal organisms, to avoid unnecessary inflammation to exogenous antigens (including food allergens) or damage-associated self-antigens, and, very importantly, to prevent the invasion or dissemination of pathogenic organisms (5–9). Remarkably, the lamina propria layer of the small intestine retains the highest concentration of

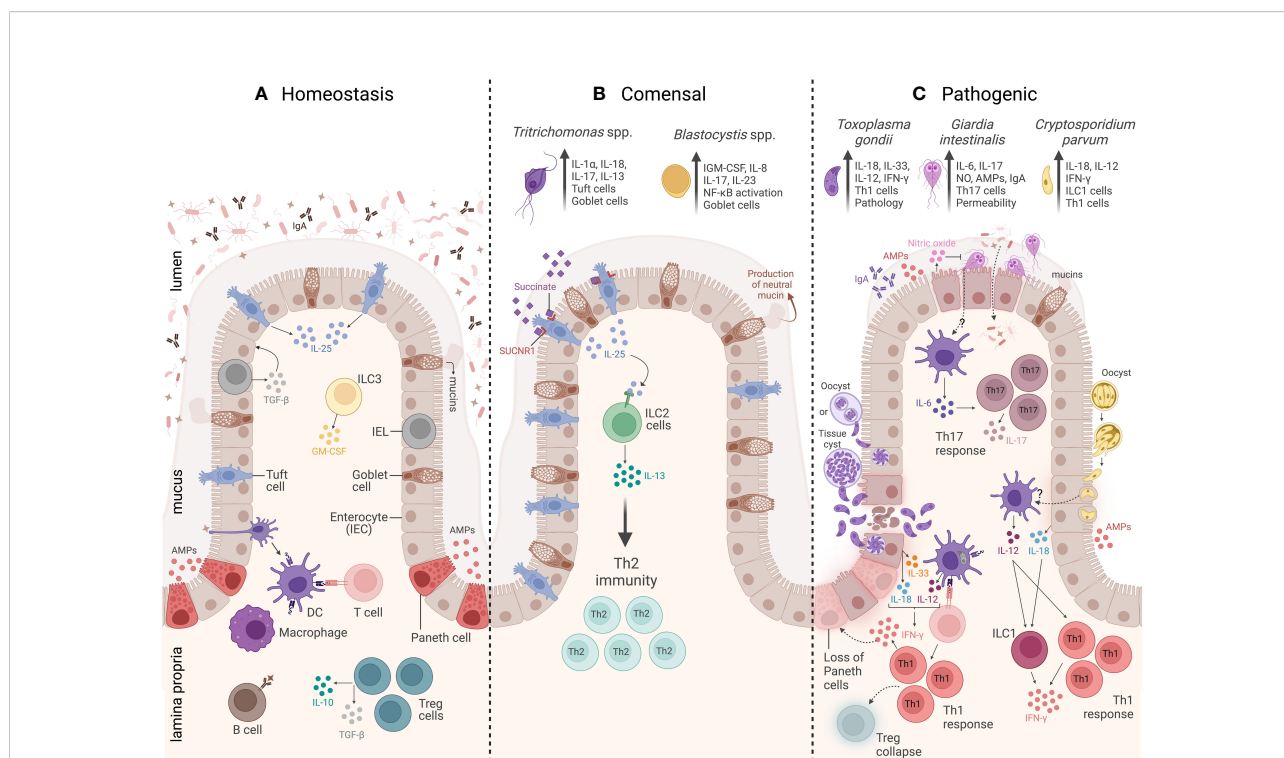


FIGURE 1

Overview of the intestinal mucosal immunity during homeostasis, commensal protozoa colonization, and pathogenic protozoa infection. **(A)** The homeostasis of a healthy intestine is maintained by several biochemical and cellular components that form a physical barrier, including a mucus layer that isolates the lumen from the epithelium and underlying the mucus. There is a group of specialized epithelial cells composed of enterocytes, goblet cells, and Paneth cells that secrete antimicrobial peptides (AMPs), tuft cells that constitutively express IL-25, and intraepithelial lymphocytes (IELs) that regulate epithelial growth through the secretion of TGF- $\beta$ 1. Underneath the epithelium is the lamina propria (LP), and the main cells involved in homeostasis are macrophages and dendritic cells (DCs) responsible for tolerance and T-cell activation. Innate lymphoid cell 3 (ILC3) is the main source of granulocyte-macrophage colony-stimulating factor (GM-CSF), while T regulatory cells (Tregs) are key to maintaining intestinal balance and tolerance through the secretion of IL-10 and TGF- $\beta$ . **(B)** The commensal protozoa colonization is represented by two protists, *Trichomonas* spp. and *Blastocystis* spp. In the small intestine, *Trichomonas* spp. secrete succinate molecules that bind in tuft cells SUCNR1 receptors, inducing these cells to release IL-25 resulting in ILC2 activation and IL-13 secretion, leading to Th2 response and goblet cells hyperplasia. Meanwhile, in the large intestine, *Trichomonas* spp. induce Th1 and Th17 immune responses by eliciting IL-1 $\beta$ , IL-18, and IL-17 cytokine release. *Blastocystis* colonization induces goblet cell hyperplasia, which leads to neutral mucin production and stimulates Th1/Th17 immune response, with IL-17 and IL-23 cytokine signatures. **(C)** The intestinal mucosal immunity to pathogenic protozoa infection is represented by three parasites: *Toxoplasma gondii*, *Giardia intestinalis*, and *Cryptosporidium parvum*. *T. gondii* infection is characterized by a strong Th1 immunity, tissue damage, and immunopathology. The immune response to this protozoan is characterized by high levels of IL-33- and IL-18-producing epithelial cells, as well as IL-12 production by DCs. All these three cytokines act together to induce CD4<sup>+</sup> T cells producing IFN- $\gamma$ . The infection is also characterized by Treg collapse and a loss of Paneth cells due to the high levels of IFN- $\gamma$ . *G. intestinalis* infection is characterized by a Th17 immunity, elicited by IL-6-producing DCs and IL-17-producing CD4<sup>+</sup> T cells. In addition, *G. intestinalis* increases intestinal epithelial permeability and microbial translocation, as well as enhances IgA, AMPs, nitric oxide (NO) levels, and mucin secretion. *C. parvum* infection is characterized by a Th1 immune response, which is associated with secretion of AMPs and IL-18 by epithelial cells, IL-12 by DCs, and IFN- $\gamma$  production by both innate lymphoid cells 1 (ILC1) and CD4<sup>+</sup> T cells. Created with BioRender.com.

immune cells, which mediate host protection against infective agents and also promote bystander inflammation and pathology (1).

Anatomically, the intestinal mucosa is composed of an epithelium, which consists of a folded single layer of epithelial cells, connected by tight junctions, that is superimposed by a mucus layer (10). It is organized into crypts (invaginations into the underlying mesenchyme) and villi (projections into the lumen) (11). The cellular composition of the epithelium includes enterocytes, goblet cells, Paneth cells, M cells, tuft cells, enteroendocrine cells, and intraepithelial lymphocytes (IELs) (12–14). Underlying the epithelium is the lamina propria (LP), formed by structural elements like fibroblasts, fibrocytes, vascular endothelial cells, muscle cells, and immune cells like macrophages, mast cells, dendritic cells (DCs), plasma cells, and B and T cells, which are responsible for maintaining homeostasis, immune tolerance to commensals, and a defensive barrier against invasive pathogenic agents (1, 13). The epithelium thus represents both a physical barrier and an important site for the production of cytokines, chemokines, and antimicrobial peptides that protect the host against various infections and danger signals (Figure 1A).

Epithelial cells are comprised of a diverse array of specialized cell types, which include goblet cells (professional mucin-producing cells responsible for forming a dense mucus gel layer) that function to protect and maintain healthy epithelial function; tuft cells are immune sentinels and taste-chemosensory cells that constitutively express IL-25; Paneth cells are important sources of antimicrobial peptides (AMPs; defensins and lysozyme) and growth factors, which are associated with host protection against enteric infections, maintenance of crypt stem cell activity, and intestinal homeostasis by their ability to regulate the microbiome in the lumen (11, 15–19). Finally, the IELs, composed of  $\gamma\delta$  T cells and CD8<sup>+</sup> T cells (expressing the CD8 $\alpha$  homodimer), are interspersed throughout the intestinal epithelial layer and regulate epithelial growth and homeostasis by their secretion of TGF- $\beta$ 1 (20). IELs do not require priming to respond since they are antigen-experienced T cells that release cytokines and kill target cells upon encountering specific antigens (21, 22).

In the lamina propria, macrophages, DCs, innate lymphoid cells (ILCs), natural killer (NK) cells, and T and B lymphocytes are the immune cells that work together to maintain intestinal mucosal homeostasis and barrier integrity. The myeloid compartment, represented by macrophages and DCs, is crucial for the maintenance of intestinal tolerance and the activation of T-cell immunity (23, 24). The recently described innate immune cell family of ILCs within the intestinal mucosa is comprised of three different groups, namely, ILC1, ILC2, and ILC3. Of note, ILC3s are critically involved in intestinal homeostasis and consist of the primary source of granulocyte-macrophage colony-stimulating factor (GM-CSF) (25–27). Intestinal microbes, by their ability to promote B-cell receptor (BCR)

editing and the immunoglobulin diversification of resident B cells, also play an important role in the induction of tolerance against commensal antigens in the mucosa (28, 29). The intestinal mucosa is likewise home to the largest number of T cells in the body, comprised of a heterogeneous array of CD4<sup>+</sup> and CD8<sup>+</sup> T cells (29, 30). Most of these T cells in the mucosa possess an activated/memory phenotype, and their activation culminates in a rapid proliferative response (31). Among the most enriched T-cell populations in the gut are regulatory T cells (Tregs), which promote the maintenance of immune intestinal balance and tolerance, mainly by the secretion of IL-10 and TGF- $\beta$  (32).

Understanding the orchestration and characteristics of the mucosal immune response during colonization by pathogenic or commensal organisms within the intestine may provide novel insights into the mechanisms underlying microbe-induced protection and/or immunopathology. Of note, most of our knowledge about the interactions between the host mucosal response and the gut microbiome, including the eukaryome, is dominated by studies focused on bacterial communities, helminth parasites, and intestinal viruses. Importantly, the impact of commensal and pathogenic eukaryotic protists that colonize the intestinal mucosa is less studied. In this review, we provide an overview of the current knowledge of protozoan-induced intestinal mucosal immunity, specifically, how commensal and pathogenic protozoa trigger the development and modulation of innate and adaptive immune responses in experimental models of infection and what the pathological consequences are for the host, if any.

## Mucosal immunity to commensal intestinal protozoa

Investigating the host gastrointestinal immune response to the microbiome is an emerging field. Most studies are focused on host responses generated against enteric bacterial communities. However, recent advances in amplicon-based next-generation sequencing (NGS) and metagenomics analysis have allowed a more comprehensive identification and description of the diverse micro-eukaryotes living within the mammalian microbiota, such as the fungi and protozoa that comprise the eukaryome (33). Large and small intestine-colonizing eukaryotes are commonly associated with pathogenic organisms such as *Toxoplasma gondii*, *Giardia* spp., and *Cryptosporidium* spp., which are discussed further in the second part of this review. However, enteric non-pathogenic commensal protozoa have also been identified by their ability to alter the gut-bacteriome diversity and to prime the host-immune response (34). Currently, relatively few investigations have examined gastrointestinal tract (GIT) immunity generated by the presence of protozoa that exist as enteric commensals. In this

review, we specifically focus on *Blastocystis* spp. and *Tritrichomonas* spp. (Figure 1B).

## Blastocystis spp.

As reviewed by Parija and Jeremiah, *Blastocystis* spp. were first described by Brittan and Swayne during the 1849 cholera epidemic in London. To this day, the taxonomic classification of this organism remains controversial in the scientific community (35). In the early 1900s, Alexeieff and Emile Brumpt classified *Blastocystis* as a harmless saprophytic yeast of the intestinal tract. However, in 1967, Zierdt *et al.* reclassified the organism as a protist based on its morphology and phenotypic properties (36). Thus, after various observations over the decades, *Blastocystis* is currently classified as an anaerobic, non-invasive protist that colonizes the mammalian intestinal tract and belongs to the Stramenopiles, a clade of protists that group with algae, diatoms, and water molds (37). It possesses a high genetic diversity and a vast host range, including livestock and humans (38). Epidemiological estimates suggest that more than 1 billion people worldwide are colonized with *Blastocystis* spp., varying at 0.5%–23.1% in developed countries versus 22.1%–100% in resource-limited countries (39–41). *Blastocystis* spp. is currently comprised of 28 subtypes (STs), 12 of which are reported to colonize the GIT of humans (ST1–ST8, ST10, ST12, ST14, and ST16), but among them, only ST1–ST4 account for more than 90% of the human infections reported globally, whereas ST5–ST8, ST10, ST12, ST14, and ST16 are rarely found in human stool (42–46). *Blastocystis* has been detected in the human enteric microbiota in both healthy and disease conditions by different methods such as real-time PCR, amplicon-based NGS, and metagenomics. Its presence within the gut microbiota is associated with a shift in bacterial composition (47). The association between *Blastocystis* and the shift in microbial diversity and composition, as well as its effects on the host response remain to be determined. Although reports of this parasite are becoming more common in human gut studies, the direct *Blastocystis* mucosal immune response in the murine model is still under-explored.

Whether *Blastocystis* is pathogenic under some circumstances, for example, during host immune suppression, remains unclear, largely because screening for the presence of other known microorganisms such as viruses, protozoa, or bacteria has not been systematically performed (48, 49). Some studies have described a positive correlation between *Blastocystis* colonization and conditions such as irritable bowel syndrome or inflammatory bowel disease in humans (50, 51), whereas the high prevalence of this protist observed in healthy human guts has fueled a separate debate on whether this parasite is non-pathogenic and a commensal of the human GIT. Indeed, a sample cohort of healthy humans in Ireland identified that 56% of individuals were positive for *Blastocystis* by 18S rRNA PCR (52).

*In vitro* models suggest that *Blastocystis* can regulate host immune responses. Colonic epithelial cells exposed to *Blastocystis* Nand II strain released IL-8 and GM-CSF cytokines (53), possibly triggered by *Blastocystis* cysteine proteases, which are released by the zoonotic isolate WR1 (ST4) and activate the NF- $\kappa$ B transcription factor (54). In addition, *Blastocystis* ST7 (B isolate) can evade the immune response by downregulating inducible nitric oxide synthase (iNOS) (55) and degrading human secretory immunoglobulin A (IgA) (56).

Iguchi *et al.* determined that *Blastocystis* RN94-9 strain (a subtype 4 isolate from a laboratory rat) is not inconspicuous. Although rats experimentally infected with ST4 RN94-9 cysts largely failed to display pathogenic markers during colonization, such as weight loss, diarrhea symptoms, mucosal epithelium lesions, or inflammatory cell infiltrates (57), they did, however, induce type 1 proinflammatory cytokines such as IFN- $\gamma$ , IL-12, and TNF- $\alpha$  and cause a mild goblet cell hyperplasia that was associated with an increase in the production of neutral mucins (57). Goblet cells are essential cells for maintaining gastrointestinal homeostasis. They synthesize neutral and acidic mucins to produce and sustain the enteric mucus layer, an innate barrier against pathogens (58). Alterations in the neutral and acidic mucin ratio have also been detected in rats infected by the parasitic nematode *Nippostrongylus brasiliensis* (59). While this imbalance in the production of neutral versus acidic mucins is thought to change mucus layer charge, whether it influences parasite-mucosa attachment is less clear.

In addition to the activation of a Th1 cytokine signature, *Blastocystis* modulates the *in vivo* Th17 cytokine immune response. Th17 immunity is strongly correlated with the clearance of extracellular parasites, inflammation, and auto-immune diseases (60). IL-17, IL-23, and IL-22 cytokines have a prominent role in Th17 subset activation (61). BALB/C mice infected with *Blastocystis* spp. developed significant increases in their IL-17 and IL-23 levels in the intestinal mucosa (62). In summary, *Blastocystis* appears to modulate the enteric host immune response by inducing specific Th1/Th17 cytokine production. Hence, colonization by *Blastocystis* may generate a level of bystander inflammation that could conceivably protect mice from pathogenic infections or make them more susceptible to certain auto-immune conditions. Therefore, studies to understand the kinetics, level of, and persistence of cytokine induction during *Blastocystis* colonization are needed. In addition, the data thus far investigating *Blastocystis* colonization *in vivo* certainly argue for the necessity to screen for the presence of commensal parasites in the gut in experimental workflows in order to gain a better understanding of their role in gastrointestinal disease models.

## Tritrichomonas spp.

*Tritrichomonas* spp. comprise a complex group of neglected gut-dwelling protists that have similar morphology but possess



extant diversity. Studies within this group of parasites are limited, largely because of a lack of high-quality reference genomes or methods for axenic culture. Closely related species such as *Tritrichomonas muris*, *Tritrichomonas musculus*, and *Tritrichomonas rainier* have been discovered recently as common commensal protists that establish persistent, long-lived infections that impact the microbiome and mucosal immune homeostasis within the intestinal tract of wild and laboratory mice (63). Colonization of mice with these protists shows little to no evidence of epithelium lesion, blood cell infiltration, or other detectable gut-related disease symptoms, e.g., diarrhea, weight loss, or lethargy. Colonized mice do experience, however, a mild goblet cell hyperplasia and display significant changes in their mucosal immunity, which protect them from GIT bacterial infections but increase their susceptibility to inflammatory bowel-like diseases (64, 65). These studies certainly highlight the importance of screening the microbiota of laboratory mice for *Tritrichomonas* commensal infection.

In the healthy gut under homeostatic conditions, immune sentinels and taste-chemosensory cells called tuft cells are commonly found in low numbers within the epithelia of the small and large intestine in mice. However, in response to various parasitic infections, tuft cells are known to expand exponentially. For example, enteric non-pathogenic commensal protists, including *Tritrichomonas* spp., and pathogens such as nematodes (e.g., *N. brasiliensis*, *Heligmosomoides polygyrus*, and *Trichinella spiralis*) and trematodes (e.g., *Echinostoma caproni*) cause tuft cell activation and expansion, triggering mainly type 2 host immunity involving ILC2s (66–68).

Howitt *et al.* first described markedly different intestinal tuft cell numbers between specific pathogen-free BIH mice bred “in-house” versus BIH mice purchased from the Jackson Laboratory (JAX). When cecal contents from BIH mice were fed to the JAX mice, intestinal tuft cells proliferated to a level identified in the bred “in-house” BIH mice, and the transmissible component responsible for the phenotypic change was a single-celled protist that they referred to as *T. muris* (65). Two other contemporaneous studies likewise concluded that this and a related gut-dwelling commensal protist referred to as *T. musculus* alter immune cell homeostasis within the gut microenvironment, conferring the protection of colonized mice from mucosal bacterial infections at the expense of increasing their susceptibility to colitis and colorectal tumors (60, 67).

The Howitt study showed that shortly after *T. muris* colonization, tuft cells release the cytokine IL-25 and promote ILC2 activation and the secretion of IL-13, resulting in goblet cell hyperplasia and skewing the immune potential of the gut toward a Th2 response (65). Two years later, Nadsombati *et al.* showed that the related parabasalid *T. rainier* also induced tuft cell expansion, ILC2 activation, and goblet cell hyperplasia in the small intestine of laboratory mice, similar to *T. muris* (69). Their work established that succinate secreted by *T. rainier* is a pivotal

ligand sensed by the succinate receptor (SUCNR1) expressed on tuft cells and that this was sufficient to initiate a Th2-biased immune response in the small intestine (69). Succinate is a metabolite produced by hydrogenosomes, a mitochondria-like organelle that synthesizes ATP in anaerobic protists, including *Tritrichomonas* spp (70). This remarkable finding revealed for the first time that a metabolite unique to a eukaryotic microbe was able to directly activate type 2 immune cell responses *via* a tuft cell-ILC2 sensing and activation circuit (19).

Paradoxically enteric *Tritrichomonas* spp. infection also activates Th1 and Th17 immune cells and their signature cytokines during mouse colonization, in addition to initiating Th2-biased immune responses in the small intestine. In the large intestine specifically, *T. muris* stimulates a Th1-biased pro-inflammatory response, increasing IL-12/IL-23p40 and IFN- $\gamma$  proteins to levels that exacerbate colitis in Rag1<sup>-/-</sup> mice post-adoptive T-cell transfer (71). Chudnovskiy *et al.* also demonstrated a CD45<sup>+</sup> hematopoietic cell expansion and the secretion of high levels of IgA in the large intestine of mice colonized with *T. musculus* (*T.mu*), a *T. muris*-related species (64). Like *T. muris*, *T.mu* also induced mild goblet cell hyperplasia and an exacerbated inflammatory response in colitis and tumorigenesis models. Within the cecal epithelium, high levels of IL-18 were released, the result of activating an ASC-dependent inflammasome, and this was important for the induction of colonic Th1 and Th17 immune responses in *T.mu*-colonized mice (64). The altered inflammatory state driven by the IL-18 release within the colonic epithelium was sufficient to protect mice from *Salmonella typhimurium* enterocolitis and established that the presence of *T.mu* significantly increased anti-bacterial defenses within the enteric mucosa (64). Recent work has identified that *T.mu* activates both the NLRP1B and NLRP3 inflammasomes and also causes a dramatic increase in luminal extracellular ATP levels (72). *T.mu* colonization likewise triggered IL-13 by ILC2s in the mucosa, as previously reported by Howitt *et al.* using *T. muris* (65). It also altered host glucose homeostasis by increasing gluconeogenesis, as well as increasing free choline production in the gut of infected hosts. The parasite metabolite that triggered this pathway is unknown, but there is evidence for a succinate-independent process (73). Altogether, the data described here emphasize the importance of investigating commensal protists in the gut microbiota in order to devise new therapeutic interventions to control inflammatory, pathogenic, and/or metabolic diseases.

## Intestinal mucosal immunity to pathogenic protozoa

A healthy and functional intestinal mucosa is both plastic and dynamic, which is necessary to maintain immune homeostasis and protect hosts from invading pathogenic

organisms. However, dysregulated immune responses that result in chronic diseases within the gut epithelia have negative consequences for the host, including intestinal damage and a failure to control microbial pathogens (3). The following section summarizes the current knowledge on the intestinal mucosal immune response to the pathogenic protozoan parasites *T. gondii*, *Giardia intestinalis*, and *Cryptosporidium parvum* and describes the most relevant epithelial and immune cell populations involved in such responses (Figure 1C). Although these are all human parasites and their associated diseases are extremely relevant within the human population globally, our focus in this review is to describe the major features of the mucosal immune response triggered by these intestinal protozoa in experimental animal models to better understand the biology of their parasitism and highlight the parasite and host factors that specifically influence their pathogenicity.

## Toxoplasma gondii

*T. gondii* is an intracellular protozoan parasite capable of infecting essentially any warm-blooded animal. It is highly prevalent worldwide, with about 1/3 of the human population chronically infected with *T. gondii* parasites (74). The parasite is a leading cause of infectious blindness and is considered a life-threatening disease among the immunocompromised. It is also capable of causing abortion or birth defects during congenital infection (75, 76). *Toxoplasma* transmission occurs by oral ingestion of infectious tissue cysts or oocysts; hence, intestinal mucosal immunity constitutes the first line of defense and is one of the most important barriers against *T. gondii* (13, 77, 78). In the small intestine, *T. gondii* differentiates into tachyzoites, the rapidly replicating form of the parasite, which ultimately disseminates infection beyond the intestinal mucosa, colonizing other tissues, such as skeletal muscle and the central nervous system, before it differentiates into the bradyzoite form, which establishes latent infection in the form of tissue cysts (13). When in the gut, *Toxoplasma* alters the mucosal homeostatic balance and induces a cascade of immunological events involving components of both innate and adaptive immunity, characterized by a polarized Th1 immune response that induces high levels of IFN- $\gamma$  and a collapse of Tregs (13, 79). The heightened inflammatory state has been shown to cause a Crohn's disease-like enteritis, the result of an IFN- $\gamma$ -mediated acute ileitis that occurs in some inbred mice (77, 80–83). Type 1 immunity is normally associated with host protection and is necessary for *T. gondii* clearance. However, in some *Toxoplasma* murine infection models, a dysregulated Th1 response can occur, which causes irreversible pathological alterations and inflammatory responses that promote severe tissue damage and host death (82–84). Here, we describe the mucosal-associated immune cell types that are

induced during *T. gondii* infection in the gut with reference to well-described parasite factors that regulate the development of the *T. gondii*-associated mucosal immune response.

Gut epithelial and lamina propria cells are key players in host defense against *Toxoplasma* infection in the intestine. The gut epithelium is an important physical barrier and functions as the first line of innate defense against the parasite. In response to oral *Toxoplasma* infection, the gut epithelium releases alarmins IL-18 and IL-33 (both IL-1 family members), which synergize with IL-12 to promote protective IFN- $\gamma$ -mediated immune responses that regulate *T. gondii* infection within the ileum. The release of IL-18 is caspase-1/11, ASC, and inflammasome sensor NLRP3- and NLRP1b-dependent (85). IL-18 plays a pivotal role in limiting parasite replication, as mice deficient in IL-18 or IL-18R experience 20–100-fold higher parasite loads and die acutely, compared to infected wild-type mice with the same genetic background (85, 86). It has also been reported that *T. gondii* actively crosses the gut epithelial barrier by invading, replicating, and lysing epithelial cells (78, 87). This process ruptures the gut epithelium and releases the damage-associated molecular pattern (DAMP) cytokine IL-33, which also synergizes with IL-12 to promote ILC1 production of IFN- $\gamma$  and CCR2-dependent recruitment of inflammatory monocytes required for resistance to *T. gondii* (88). *T. gondii* tachyzoites are also released into the lamina propria compartment. Such collateral damage facilitates the translocation of bacteria across the gut epithelium, which exacerbates the inflammatory response and causes serious gut pathology (78, 89–92).

The presence of IFN- $\gamma$  driven by *T. gondii* infection is also responsible for the rapid loss of Paneth cells within the host mucosa and results in a myriad of pleiotropic effects. Paneth cells are epithelial cells that secrete a suite of antimicrobial proteins and peptides in order to sustain mucosal homeostasis (15). In the presence of *Toxoplasma*, alterations in both membrane and mitochondrial integrity occur in Paneth cells which activate an mTORC1-dependent cell death pathway (93–95). The loss of Paneth cells is also partially associated with the activation of TLR11 signaling in the gut (93). Likewise, IELs, another epithelial cell type comprised mainly of CD8 $\alpha$  T cells, play a critical role in the initiation of tissue damage and immunopathology in response to *Toxoplasma* infection. The progression of pathological inflammation and necrosis is associated with the expansion of CCR2<sup>hi</sup>CD103<sup>+</sup> IELs in the epithelium, which are principally induced by the presence of the CCL2 chemokine. Interestingly, CCR2<sup>-/-</sup> mice experience diminished intestinal inflammation but had higher mortality rates, due largely to a failure to regulate *Toxoplasma* growth (96). Hence, IELs perform a critical function. Failure to regulate them precisely represents a double-edged sword: IELs secrete anti-inflammatory mediators, like TGF- $\beta$ , to regulate mucosal inflammation in the GIT but can also expand to produce high levels of IFN- $\gamma$ , which drives the immunopathology in response to *Toxoplasma* infection (77).

After crossing the epithelial barrier, parasites reach the LP, where immune cells are abundantly present. In the LP, innate immune cells, including inflammatory monocytes, dendritic cells, and NK cells, are critical players that both initiate and regulate the immune response against *T. gondii* infection (76). The myeloid compartment, specifically macrophages and dendritic cells, is responsible for sensing parasite antigens (pathogen-associated molecular patterns (PAMPs)) and rapidly respond by secreting IL-12, one of the major cytokines released in response to *Toxoplasma* infection. IL-12 principally drives the marked IFN- $\gamma$  production by NK and T cells (97, 98). Among innate immune cells of non-myeloid origin, the role of ILCs during *T. gondii* infection is not well defined, so it remains unclear precisely how they contribute to the induction or regulation of mucosal immunity against *Toxoplasma*. Although it is known that ILC1 cells can secrete IFN- $\gamma$ , the contribution of this cell type to the total aggregated IFN- $\gamma$  levels during *Toxoplasma* infection is likely less relevant than that of CD4<sup>+</sup> T cells. Indeed, López-Yglesias *et al.* demonstrated that in the absence of ILC1, Tbx21<sup>-/-</sup> mice were still able to generate a robust IFN- $\gamma$  response driven by CD4<sup>+</sup> T cells (94). Of note, Wagage *et al.* suggested that RORyt<sup>+</sup> ILC3 cells limit T-cell responses and pathology during *T. gondii* infection; however, they showed that ILC3 frequency is significantly decreased in the presence of the parasite (99). More studies on ILCs are required to fully understand their role in oral *T. gondii* infection models. Likewise, the role of neutrophils, another innate immune cell of myeloid origin, has not been systematically studied, so its role in contributing to mucosal immunity during *Toxoplasma* infection is not clear. Neutrophils rapidly migrate into the lamina propria during acute infection and can be detected within 3 days post-oral inoculation of *T. gondii* cysts (100, 101). Coombes *et al.* reported that tachyzoites preferentially infect infiltrating neutrophils, which function as reservoirs for *T. gondii* dissemination to other host tissues (102). Further studies are necessary to define the role of neutrophils during *Toxoplasma* intestinal infection.

One of the most important and well-studied cell types responding to *T. gondii* infection is DCs. These cells are the main source of IL-12 production and are central for the activation of T cells during both intraperitoneal and oral *T. gondii* infection. Several studies previously demonstrated that *T. gondii* proteins can directly activate Toll-like receptors (TLRs) to induce IL-12 production by DCs and macrophages. These include Micronemes 1 and 4 (TLR2/TLR4 agonists) and Profilin (TLR11/TLR12 agonists) (103–107). Although most of these studies were carried out using intraperitoneal infection models, it is known that profilin and TLR11 interactions also activate the intestinal innate immune response during oral infection with *T. gondii*. Benson *et al.* showed that mice deficient in TLR11 expression are more susceptible to *Toxoplasma* infection, the result of a marked reduction in IL-12 secretion and a partial defect in the generation of Th1 cells

(108). In addition, recent studies have reported that IL-12—released during *Toxoplasma* infection—arises mainly from uninfected lamina propria DCs (CD11b<sup>-</sup>CD103<sup>+</sup>) rather than infected ones (109–111). It has been suggested that these cells respond to soluble parasite factors secreted prior to parasite invasion or parasite proteins that have been actively injected into DCs by *T. gondii* (109–111). Other parasite factors that regulate mucosal immunity include the Type II dense granule protein 15 (GRA15<sub>II</sub>), which activates NF- $\kappa$ B within innate immune infected cells and results in elevated IL-12 production, lower parasite burden, and less intestinal inflammation. This event was reported to occur only when the Type I rhoptry kinase 16 (ROP16<sub>I</sub>), another parasite factor that is involved in STAT3, STAT5, and STAT6 activation, was heterologously expressed within *T. gondii* Type II strains (112, 113). Hence, the combined expression of GRA15<sub>II</sub> and ROP16<sub>I</sub> in a Type II background strain conferred host resistance to acute oral infection by *T. gondii*. Finally, GRA24 is also released into the host cell cytoplasm to induce IL-12 production by triggering the activation of host mitogen-activated protein kinase p38 (p38 MAPK), which subsequently results in the transcription of the IL-12 gene (114–117). Additionally, different studies reported that there is a large CCR2-dependent influx of inflammatory monocytes into the lamina propria compartment during *Toxoplasma* infection, and IFN- $\gamma$ -activated inflammatory monocytes are associated with the control of parasite replication and host resistance (111, 118). These cells are capable of producing IL-12 in the gut during infection, and this helps in the recruitment of protective Th1 cells in a CXCR3-dependent manner (119). Moreover, it is also reported that inflammatory monocytes are critical for *T. gondii* clearance by IFN- $\gamma$ -dependent activation of iNOS/NOS2, the release of NO, and the activation of IRGs (immunity-related GTPases), which destroy the parasitophorous vacuole membrane (PVM) to limit parasite replication (92, 120, 121).

As mentioned before, *T. gondii* infection induces a very strong and polarized Th1 immune response, associated with a high frequency of IFN- $\gamma$ -producing CD4<sup>+</sup> T cells in murine models. Although Th1 immunity is required to control parasite replication, by inducing such a strong inflammatory response, in some mouse genetic backgrounds, it also drives a severe, and often lethal, intestinal immunopathology (81, 93, 122). López-Yglesias *et al.* demonstrated that even in the absence of T-bet, the transcriptional factor signature for Th1 cells, the IFN- $\gamma$ -producing CD4<sup>+</sup> T-cell response, is still markedly increased in Tbx21<sup>-/-</sup> mice, suggesting that the immunopathogenesis during infection can be driven by a T-bet-independent pathway (94). Additionally, the *T. gondii*-Th1-induced tissue damage in the intestinal mucosa is associated with the collapse of resident Tregs and a subsequent reduction of IL-10 levels during infection (79). This phenomenon is associated with the conversion of Tregs into Tbet<sup>+</sup> IFN- $\gamma$ -producing cells and favors the development of a Th1-driven Crohn's disease-like

immunopathology (79, 123). Finally, Rachinel *et al.* suggested that the acute ileitis driven by *T. gondii* is associated with the expression of the parasite immunodominant antigen SAG1 (surface antigen 1), which elicits a CD4<sup>+</sup> T cell-dependent lethal inflammatory process in the oral B6 mouse model (124). Although Th1 responses dominate during *T. gondii* infection, a few studies suggest that IL-17A- and IL-22-secreting Th17 cells also play an important role in maintaining persistent levels of antimicrobial peptide production, given that mice deficient in class I-restricted T cell-associated molecule (CRTAM) expression on T cells failed to control *T. gondii*-driven immunopathology and microbial translocation (125, 126).

Among all the intestinal pathogenic protozoa parasites that will be discussed in this review, it is notable that the area of study regarding *T. gondii* biology has evolved dramatically during the past few years. In addition, advances in gene editing tools and experimental models are continually contributing to our understanding of important host–parasite interactions that impact host immune responses against *T. gondii*. However, the role of many parasite factors and their interaction impacting the development of host intestinal immunity remains unknown. Ultimately, further studies are required to comprehensively elucidate the underpinnings of the protective immune response that can be elicited to revert immunopathology and confer a host-protective effect by reducing or preventing *T. gondii*-driven acute ileitis.

## Giardia intestinalis

The intestinal extracellular protozoan parasite *G. intestinalis* (syn. *Giardia duodenalis* and *Giardia lamblia*) is highly prevalent worldwide and can infect all mammals, including humans, domestic animals, and wildlife. Giardiasis is considered one of the most prevalent enteric diseases caused by a protozoan parasite, with estimates ranging from 2%–5% to 20%–30% of *Giardia*-infected people in high and low-middle-income countries, respectively. Approximately 280 million new cases are documented every year worldwide (127–129). Immunocompetent individuals typically resolve the infection spontaneously within a few weeks after exposure, and some people never develop any symptoms (130, 131). However, susceptible individuals often exhibit a myriad of different gastrointestinal symptoms, including abdominal cramps, nausea, and diarrhea (132). These clinical symptoms are frequently associated with the development of malabsorption syndrome and with children's development and growth impairment, especially in chronic and recurrent *Giardia* infections (133–135). Recent data suggest that giardiasis is one of the four main contributors to stunting in children (135–137). Transmission of *Giardia* occurs through the oral–fecal route with the ingestion of parasite cysts present in contaminated water or food (138), highlighting the importance of the intestinal

mucosal immune response triggered by *Giardia* infection, which will be discussed here.

Within the gastrointestinal environment, *Giardia* cysts release trophozoites, the parasite form that adheres to the epithelial monolayer to colonize the small intestine, especially the duodenum. Trophozoites attach to the microvilli in the epithelium to replicate and complete their life cycle, but this alters mucosal homeostasis and induces several host responses. In mice, *Giardia* infection is mainly characterized by a protective Th17 immune response, with high levels of IgA secretion, and the presence of IL-17 is associated with parasite clearance (139, 140).

The intestinal epithelium functions as a physical barrier to protect from invading pathogens reaching the deeper layers of the mucosa. Although *Giardia* trophozoites do not invade the mucosa or submucosa layers in immunocompetent organisms, many studies suggest that *Giardia* trophozoites attach to the intestinal epithelial cells (IECs) and rupture the epithelial barrier by disrupting tight junction proteins within the IECs, such as claudins and occludin, which increases intestinal permeability, facilitating the entry and spread of enteric pathogens (141–143). In addition to functioning as a physical barrier to avoid parasite invasion in the lamina propria, the intestinal epithelial layer is responsible for producing various chemicals, such as nitric oxide (NO), and antimicrobial peptides that inhibit *Giardia* replication in the epithelium (144). However, *Giardia*-produced arginine deiminase (ADI) is thought to reduce the availability of arginine in the gut, which negatively affects NO production by enterocytes (144). In addition, Stadelmann *et al.* reported that arginine consumption by *Giardia* is also associated with decreased proliferation of intestinal epithelial cells during infection (145). Epithelial Paneth cells produce and release  $\alpha$ -defensins, an AMP that, in combination with NO from nitric oxide synthase (NOS2), acts to control *Giardia* burden and eliminate infection (146). *Giardia* infection also upregulates mucus production during intestinal infection (147–149).

*Giardia* infection results in the rupture of the epithelial barrier, and this event is associated with the release of chemokines that recruit immune cells required for the protective response against *Giardia*. The epithelial disruption also promotes microbial translocation and the release of parasite antigens within the lamina propria, which induces the activation of recruited and resident myeloid cells (140, 150–152). However, studies regarding the specific role of macrophages and dendritic cells during *Giardia* infection in mice are sparse in the literature. It is known that DCs are one of the main sources of IL-6, and in the absence of this cytokine, IL-6 knockout mice fail to control *Giardia* replication during *in vivo* infections (153–155). It is reported that *Giardia* can induce a cytokine profile that is less inflammatory, and it seems that the reduction of proinflammatory cytokines is mediated by the activation of TLR2 signaling in DCs and macrophages (156, 157). Paradoxically, infection in mice that lack TLR2 is associated



with reduced parasite burden and less *Giardia*-associated pathology (156). Further studies are necessary to understand the specific role of myeloid cells during *Giardia in vivo* infection.

*Giardia* induces the recruitment of mast cells into the small intestine lamina propria, and at the site of infection, these cells degranulate and release histamines and protease-1 (MMCP-1) (158, 159). Li *et al.* described that MMCP-1 interacts with cholecystokinin (CCK), and this interaction results in more intestinal contractility, which is thought to be associated with cramps, one of the most common symptoms noted by infected people (128, 159). The literature suggests that mast cells are recruited *via* activation of the complement lectin pathway since mice lacking mannose-binding lectin 2 (MBL2) and complement factor 3a receptor (C3aR) expression are impaired in mast cell recruitment during *Giardia* infection (160). Additionally, the expression of MBL2 in the intestinal mucosa is dependent on *Giardia*-induced IL-17 (147).

As mentioned before, *Giardia* infection induces the differentiation and expansion of IL-17-producing CD4<sup>+</sup> T cells, which confer a host-protective effect by limiting parasite replication. It is reported that the control of infection is dependent on T cells but independent of Th1 and Th2 immunity (139, 140, 161). However, as is the case for many other pathogens, a protective immune response that limits the *Giardia* burden may also generate significant pathology. Known sequelae associated with *Giardia* infection in mice are accelerated intestinal transit contributing to parasite-induced diarrhea, muscle hypercontractility, mast cell activation, and cramping (158, 159, 162, 163). Lastly, activated CD8<sup>+</sup> T effector cells (CD44<sup>hi</sup> and CD69<sup>hi</sup>) mediate tissue damage and microvillous shortening, which is responsible for disaccharidase enzyme (sucrase and lactase) deficiency and malabsorption of electrolytes, nutrients, and water (156, 164). The mechanism of CD8<sup>+</sup> T-cell activation during *Giardia* infection, however, remains unclear but is thought to be influenced by the intestinal microbiota (156).

While there has been significant progress in understanding the host–parasite interaction and the immune response induced by *Giardia*, further studies are needed to determine which parasite factors specifically trigger innate immune activation, as well as the signals implicated in Th17 activation within the intestinal mucosa, and how CD8<sup>+</sup> T cells become activated to induce pathology. Finally, new studies are required to better understand the nature of the protective immune response induced by *Giardia*, which is associated with a reduced risk of developing a severe diarrheal disease in children, and whether *Giardia* parasitism may be relevant or harmful in the context of a co-infection with another enteric pathogen.

## Cryptosporidium parvum

*Cryptosporidium* spp. are epicellular protozoan parasites that colonize the gastrointestinal tract of mammals. The species *C.*

*parvum* and *Cryptosporidium hominis* cause the majority of human infections globally (165, 166). In immunocompetent individuals, cryptosporidiosis is usually a self-limiting infection, with mild (abdominal pain and diarrhea) or absent symptoms, but this parasite can cause serious diseases in immunodeficient patients leading to severe, life-threatening diarrhea (166, 167). The enteric disease is also the second leading cause of severe diarrheal illness in children under 5 years old, with an estimated 60,000 deaths per year worldwide (168). In addition, it is responsible for affecting the growth and development of children. The limited availability of therapeutic treatments and the total lack of a vaccine represent a challenge for disease prevention (168). Parasite transmission occurs through the oral–fecal route upon the ingestion of infectious oocysts (169). Among the three pathogenic protozoa discussed in this review, *Cryptosporidium* infection and its associated mucosal immunity are much less explored by the scientific community, mainly because of a lack of experimental tools, including the ability to readily cultivate the parasite life cycle in the laboratory and the limited availability of animal models. Nevertheless, recent advances adapting *Cryptosporidium* to mice are facilitating new insight and permitting investigation of the intestinal mucosal immune response during *in vivo* infection.

Within the intestinal lumen, released sporozoites adhere to the epithelial plasmalemma to form a parasitophorous vacuole, a compartment isolated from both the lumen and host cell cytoplasm where parasites replicate and promote colonization of the small intestine. Cryptosporidiosis is characterized by crypt hyperplasia, villous atrophy, and diffuse shortening or loss of brush border microvilli, which results in malabsorption, dehydration, and diarrhea (166, 167). *Cryptosporidium* initiates an inflammatory process by activating epithelial cells (IECs) and inducing the secretion of “alarmin” cytokines (IL-8, TNF- $\alpha$ , and IL-1 $\beta$ ) and chemokines (CCL2, CCL5, CXCL1, CXCL8, CXCL9, CXCL10, etc.) (170, 171). This event triggers the recruitment of effector immune cells to the site of infection and inhibits parasite adhesion to the mucosal epithelium (169–172). Various other experimental studies have concluded that production of  $\beta$ -defensin antimicrobial peptides, as well as cytokines, chemokines, and prostaglandin E2 by IECs, occurs *via* activation of the TLR2/TLR4/NF- $\kappa$ B signaling pathway during *Cryptosporidium* infection (171, 173, 174). Moreover, NF- $\kappa$ B activation induces the expression of anti-apoptotic factors that prolongs the life of IECs, promoting parasite replication and the propagation of the infection (175, 176).

The secretion of chemokines by *Cryptosporidium*-activated IECs is responsible for the recruitment of inflammatory monocytes, macrophages, and NK cells to the site of infection, and the presence of local IL-18 and IL-12 induces a synergistic activation of macrophages and NK cells to secrete high levels of IFN- $\gamma$  in infected neonatal mice (170, 177). Depletion of NK cells was associated with an exacerbated infection in neonatal mice (178). Furthermore, Choudhry *et al.* reported that the

neutralization of IL-18 during *Cryptosporidium* infection, after *in vivo* treatment with anti-IL-18 antibodies, resulted in decreased IFN- $\gamma$  expression in Rag2<sup>-/-</sup> $\gamma$ c<sup>-/-</sup> mice (177). Different studies using transgenic mice have shown that deficiencies in either Th1 cytokines, such as IL-12, IL-18, and IFN- $\gamma$ , or inflammasome components (caspase-1) are associated with a more susceptible or sometimes lethal phenotype to *Cryptosporidium* infection (179–183). Additionally, a recent study has demonstrated that *Cryptosporidium* is recognized by the inflammasome sensor NOD-like receptor family pyrin domain containing 6 (NLRP6), which activates enterocytes to release bioactive IL-18 and is required for parasite control (183). Finally, the synergistic secretion of IL-18 and IL-12 by activated enterocytes stimulates ILCs to produce IFN- $\gamma$ , and this event promotes parasite control by enterocytes (184). Thus, the combination of IL-12, IL-18, and IFN- $\gamma$  as well as inflammasome activation seem to be associated with a protective mechanism against infection.

Given the fact that IFN- $\gamma$  suppresses *Cryptosporidium* infection and controls parasite replication, it has been described that CD4<sup>+</sup> T cells are also essential for parasite elimination and the establishment of an effective immune response following infection (166). Many studies have reported that neutralization of IFN- $\gamma$  in Rag2<sup>-/-</sup> or SCID mice was associated with increased *Cryptosporidium* burden, persistent diarrhea, and progress of intestinal pathology, sometimes leading to mouse death (179, 185–189). Intriguingly, McDonald *et al.* showed evidence that during early *Cryptosporidium* infection, mice secreted intestinal IL-4. They showed that susceptibility to infection was increased and associated with higher production of oocysts when mice were treated with anti-IL-4 antibodies. Their data suggest that early IL-4 can promote a protective, Th1-mediated mucosal immune response that inhibits parasite development (190). The concomitant neutralization of IL-4 and IL-5 was also shown to increase oocyst shedding in infected mice (191). Lastly, the role of CD8<sup>+</sup> T cells and B cells during *Cryptosporidium* infection is less clear. Although it has been reported that they expand during infection, whether they are essential for the clearance of infection remains enigmatic (192, 193). Additionally, while parasite-specific IgM and IgG levels have been shown to increase during infection, they do not prevent the host from secondary infection, although specific antibodies are known to reduce oocyst shedding in a reinfection event (194, 195).

Despite the difficulties of conducting experimental studies in the *Cryptosporidium* field, knowledge is quickly evolving given the development of new technologies and lab strategies to improve parasite culture, life cycle, and experimental models. These new techniques are allowing previously unanswered questions about the biology of *Cryptosporidium* parasitism to be explored, including how parasite proteins can be delivered

directly to the cytosol of infected host cells (196). Still, many questions remain unanswered, such as what parasite factors are recognized by the host cells that initiate the innate mucosal immune response or what extracellular or intracellular receptors are involved in parasite recognition.

## Microbial dysbiosis: A common feature between commensal and pathogenic protozoa

The identification of the gut eukaryome, including some intestinal protozoa discussed in this review, has allowed scientists not only to characterize their interaction with the mammalian host and their capacity to re-shape intestinal immunity but also to investigate their association with a large group of bacterial communities that inhabit the mammalian gut and specifically how these interactions might alter host gut homeostasis and promote disease pathology (33, 64).

Apart from the ability to sense intestinal immunity, all enteric protozoa discussed in this review share a mutual capacity to alter the host gut microbiome. These microeukaryotes, independent of their pathogenic or commensal status, alter the diversity of the microbiome during colonization of the intestine, which can alter mucosal immune homeostasis and promote diseases (34). For instance, recent studies suggest that colonization with *Tritrichomonas* is associated with decreased microbial diversity (197), whereas colonization with *Blastocystis* increases microbial diversity and is associated with the expansion of Clostridia and depletion of Enterobacteriaceae (198), which reduces inflammation by promoting a more healthy gut microbiota. However, some enteric protozoa, such as *Toxoplasma* (91, 92, 199), *Giardia* (200–202), and *Cryptosporidium* (203, 204), promote a shift in microbial community structure associated with dysbiosis, which exacerbates diseases and facilitates bacterial translocation (205). For example, *T. gondii* causes a profound expansion of Enterobacteriaceae (especially *Escherichia coli*), resulting in dysbiosis and increased immune-driven pathology in C57BL/6J mice (92). Additionally, Wang *et al.* showed that host-derived nitrate generated by the activation of the immune response against *T. gondii* promoted the overgrowth of Enterobacteriaceae. This occurs due to the activation of the IFN- $\gamma$ /STAT1/iNOS pathway in macrophages, which is the same pathway associated with parasite control during infection (92). Indeed, it has been shown that the impaired activation of macrophages, by the absence of STAT1 signaling or deficiency in CCR2 expression or by blocking TNF- $\alpha$  with neutralizing antibodies, can induce a marked reduction of Enterobacteriaceae expansion (90). Interestingly, the influence of enteric protozoa

on the gut microbiome may also be regulated by host genetic background, because BALB/c mice infected with *T. gondii* do not develop enteric dysbiosis, nor do they promote nitrate-dependent overgrowth of Enterobacteriaceae, and they do not develop a dysregulated mucosal immune response and lethal ileitis, as seen in C57BL/6J mice (206–208).

Nevertheless, the common ability of intestinal commensal and pathogenic protozoa to induce microbial shifts during colonization results in different microbiomes and disease profiles, which can help to explain the divergence in the type of mucosal immune response that develops and whether a protozoan parasite is considered a commensal or a pathogen. Finally, the type of microbial shift that occurs and the mucosal immune response that develops not only help to explain how variations in disease can occur but may also determine whether the parasite is cleared, establishes a persistent infection, and/or is transmissible.

## What makes a protist commensal or pathogenic?

The distinction between a host-associated commensal (defined as a microbe eating at the same table) and a pathogen (a microbe that causes harm) is often obscure. Depending on the context, some commensals can cause diseases (i.e., *Blastocystis*), whereas some pathogens can persist without causing overt diseases (i.e., *Cryptosporidium* and *Giardia*). The late Stanley Falkow perhaps said it best, that some microbes, he termed “commensal pathogens”, can persist as natural members of the indigenous flora but possess an innate ability to cross anatomical barriers, invade tissues, or breach host defenses that ordinarily limit the survival or replication of other microbes or commensals (209). Perhaps the central question is rather how to limit these invasive properties in order to reduce disease potential. As mentioned above, many factors are likely to influence the relative pathogenicity of intestinal microeukaryotes, including 1) microbial shifts that expand or decrease specific classes of bacteria (taxa) that activate or regulate the immune response, 2) the host species and its genetic background, 3) the presence of microbe-associated molecular patterns (MAMPs) that exist as virulence factors that induce inflammation and/or pathology, or 4) the induction of chronic inflammation or granulomatous reactions to contain the infection. The microbial shift induced by pathogenic protozoa often differs from that induced by commensal protozoa. The molecular basis for this is varied; for example, the diversity of host or bacterial-associated metabolites can skew the immune response to either a regulatory profile or a more inflammatory one (210–213). Moreover, the bioproducts from enteric protozoa, such MAMPs, especially present in pathogenic microbes, can activate pathological

aspects of the host immune response, and the inability to regulate this response may cause diseases (3). For instance, many bacteria can be both commensal and pathogenic, such as *E. coli*, a gram-negative versatile bacterium that is a commensal organism of the healthy gut microbiome but, through the acquisition of virulence genes by horizontal gene transfer (HGT) and/or accumulation of mutations in the genome, can convert from a commensal bacterium to a pathogenic one capable of causing a wide range of extraintestinal diseases (214–219). Similar mechanisms are known to occur in other organisms, where many species or strains initially not pathogenic evolved as pathogens due to the acquisition of virulence genes or the expansion of multi-gene families and/or epigenetic regulation mechanisms that promote invasive properties (220, 221). In addition, pathogenicity could also be associated with the relative abundance of specific microbes in the gastrointestinal tract, where their uncontrolled growth promotes the transition of microeukaryotes into pathogenic ones no longer controlled by host immunity (222). Thus, many protists can be considered both commensal and pathogenic; what determines their relative pathogenicity is a combination of different factors that together combine to determine their virulence potential.

## Future perspectives

Throughout this review, it became clear and fascinating that studies focused on the investigation of intestinal protozoan biology have progressed extensively over the last decade. The discovery of new commensal protozoa and how they can reshape the host intestinal immune response has been extremely important in understanding the alteration of gut–bacteriome diversity and how this can affect the priming of mucosal immunity in general, which ultimately impacts (or defines) how the host will respond to gastrointestinal insults, such as parasitic infections or inflammatory diseases. Nevertheless, further studies are required to characterize these mechanisms in more detail and shed important insight specifically on how intestinal protozoa reshape intestinal immune potential in naïve hosts. Along with the discoveries about commensal protozoa, the advances in gene editing tools and experimental models are continually contributing to our understanding of the host–parasite interaction that impacts host mucosal immune responses against pathogenic protists. However, the precise parasite factors and how they trigger host intestinal immunity remain understudied. This review has discussed our current understanding of the intestinal mucosal immune homeostatic landscape and how it shifts in response to colonization by commensal or pathogenic protozoa. We believe that future work in this area will continue to shed important perspectives on the mechanisms underlying microbe-induced protection

and/or immunopathology and will identify new biomarkers for therapeutic intervention to clear parasites and control disease.

## Author contributions

Conceptualization: AS-S. Funding acquisition: MG. Writing – original draft: AS-S and EA-F. Writing – review and editing: AS-S and MG. All authors contributed to the article, read, and approved the submitted version.

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## Conflict of interest

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