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Review article

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# Immunity to *Cryptosporidium*: insights into principles of enteric responses to infection

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## Abstract

*Cryptosporidium* parasites replicate within intestinal epithelial cells and are an important cause of diarrhoeal disease in young children and in patients with primary and acquired defects in T cell function. This Review of immune-mediated control of *Cryptosporidium* highlights advances in understanding how intestinal epithelial cells detect this infection, the induction of innate resistance and the processes required for activation of T cell responses that promote parasite control. The development of a genetic tool set to modify *Cryptosporidium* combined with tractable mouse models provide new opportunities to understand the principles that govern the interface between intestinal epithelial cells and the immune system that mediate resistance to enteric pathogens.

## Sections



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## Introduction

For many pathogens, the gastrointestinal tract is an initial site of invasion that precedes dissemination to distal sites. In this context, the breach of the intestinal barrier allows the innate immune system to directly interface with microbial challenges and initiate the processes that culminate in the adaptive responses that resolve infection. By contrast, *Cryptosporidium* parasites are part of a select group of organisms (that includes *Cyclospora*, *Eimeria*, rotavirus, astroviruses and certain helminths) that are restricted to the intestinal tract and do not typically spread to other sites in immunocompetent hosts. As a result, it appears that these pathogens do not readily interact with local immune cells (for example, dendritic cells, macrophages or neutrophils). How these organisms trigger mechanisms of resistance is a less studied area of infectious disease biology. Enterocytes, which are tall columnar epithelial cells that make up the majority of cells in the intestinal epithelial barrier, have emerged as key players in the local dialogue between host and microorganisms. As these cells must distinguish between commensal and pathogenic microorganisms, the ability to study *Cryptosporidium* provides the opportunity to dissect the fundamental pathways that contribute to innate and adaptive resistance in the gut without systemic infection as a confounding factor.

Protozoan parasites of the genus *Cryptosporidium* have long been recognized as pathogens of animals[1], but since the 1980s, *Cryptosporidium* has gained prominence as an important cause of enteric illness in humans in various epidemiological settings[2,3]. Globally, 8.5% of deaths that occur in children under 5 years of age are attributed to diarrhoeal disease, and the Global Enteric Multicenter Study (GEMS) published in 2013 found *Cryptosporidium* second only to rotavirus as the cause of severe diarrhoea and death in infancy[4,5]. In malnourished children, infection with *Cryptosporidium* is prolonged and can be life-threatening, which is probably an indication of the link between nutritional status and immune function[6,7]. Childhood infections with *Cryptosporidium* take a massive toll on infants and toddlers and are associated with long-term deficits in growth, weight gain and cognitive development, even in the absence of overt symptoms[4,8,9]. Although epidemiological data have shown that children younger than 2 years of age are susceptible to recurrent cryptosporidiosis, by 6 years of age the number of infections declines sharply[10]. These observations indicate that repeated exposures induce long-term protective immunity, an idea that is supported by experimental studies in large animals and mouse models[11,12].

In immune-competent adults, experimental challenges with *Cryptosporidium* have demonstrated that after onset of symptoms, infection lasts 1–2 weeks, but is typically self-limiting[13,14]. However, individuals with select primary and acquired immune deficiencies fail to resolve this infection. Cryptosporidiosis was an original AIDS-defining opportunistic infection and severe *Cryptosporidium* infection has also been observed in other patients with suppressed T cell responses, such as organ transplant recipients, and individuals with primary immunodeficiencies that affect T cells[15–18]. Nitazoxanide is the only drug approved for the treatment of this infection, but it lacks efficacy in immune-compromised patients and comparisons between studies have inferred that malnourished children are less responsive to treatment with nitazoxanide than those with better nutritional status[2,19,20]. The lack of a vaccine or effective treatment options compounds the global health threat posed by *Cryptosporidium*. Furthermore, this reinforces the need to understand the fundamental mechanisms that mediate resistance to this organism to develop better strategies to prevent and manage cryptosporidiosis in vulnerable populations.

In this Review, we summarize the known mechanisms of detection and induction of innate immunity that contribute to restriction of *Cryptosporidium* infection. In addition, the processes required for activation of T cell responses that are necessary for long-term resistance and parasite clearance are discussed.

## Biology of *Cryptosporidium*

*Cryptosporidium* species are unicellular eukaryotic parasites of the phylum Apicomplexa. The main species that infect humans are the anthroponotic *Cryptosporidium hominis* and zoonotically and anthroponotically transmitted *Cryptosporidium parvum*, which has a broad host range and is an important livestock pathogen. *C. parvum* infects adult, immunocompetent mice poorly, but early studies that used mice with genetic immunodeficiencies[12,21,22] or neonatal mice that are more susceptible to infection[23] have been foundational for identifying IFNγ and T cells as crucial aspects of host immunity. More recently, *Cryptosporidium tyzzeri*, a natural pathogen of mice, has also been shown to replicate many aspects of human disease[24–26]. In addition, advances in the capacity to genetically modify *C. parvum* strains (Box 1), which are maintained through passage in IFNγ-deficient mice, has led to the generation of mouse-adapted *C. parvum* that readily infects immunocompetent hosts[27,28].

The ingestion of *Cryptosporidium* oocysts leads to the release of sporozoites into the small intestine, which then infect and replicate in intestinal epithelial cells (IECs). In some patients with severe immune deficiencies, parasites may spread to the bile ducts and gall bladder where they infect cholangiocytes, which are specialized epithelial cells in the biliary tree[29]. In addition, evidence for respiratory infection and transmission has been recently documented for *C. hominis* in children[30]. Multiple distinct secretory organelles, which include micronemes, dense and small granules, and a single rhoptry, are important for parasite motility and the ability to invade host cells[29,31]. As sporozoites invade IECs, they establish and replicate within a parasitophorous vacuole, which is distinct from the host vacuolar system[32]. *Cryptosporidium* is unique among apicomplexans in that its parasitophorous vacuole occupies a peculiar intracellular, yet extracytoplasmic niche at the apical surface of the cell[33,34] (Fig. 1). During invasion, *Cryptosporidium* builds a complex host–parasite interface that includes a ring-shaped tight junction that constrains the interface to the base of the parasite, a membranous structure known as the feeder organelle, and a pedestal of dense bands that are probably composed of parasite proteins and host-derived actin that underlies the parasite[33]. Early events associated with invasion also include the injection of proteins from the parasite rhoptry into the IEC that remodel the host cell cytoskeleton[31]. The ability to induce actin pedestals in IECs is a conserved strategy for pathogens at the apical side of IECs and is also associated with the extracellular bacteria *Citrobacter rodentium* and enteropathogenic *Escherichia coli*[35].

Once inside its host cell, parasites replicate by merogony[36], in which three rounds of DNA synthesis and nuclear division occur without cytokinesis, leading to a multinucleated developmental stage (Fig. 1). Synchronous budding then yields eight motile stages (merozoites) that egress from the host cell to infect neighbouring IECs (Fig. 1). After three such cycles of asexual amplification, differentiation into male or female gametes occurs[37,38]. The male programme generates small, non-flagellated cells that are released into the gut lumen to find and fertilize the intracellular females in the epithelium, which leads to genetic recombination, meiosis and oocyst formation (Fig. 1). Each intracellular replication cycle takes approximately 12 hours[38],

and oocysts shed in the faeces can be detected 2–3 days after initial infection[39]. A substantial proportion of oocysts will also hatch within the same host and sustain the infection. Thus, the entire *Cryptosporidium* life cycle occurs within a single host and, if unchecked by immune mechanisms, there is tremendous potential to amplify and sustain parasite levels associated with disease.

## The small intestine as a site of infection

The commensal microbiota has a well-established role in gut biology and impacts positively on health and nutrition, but these organisms have the potential to drive aberrant inflammation. As such, many features of host–microbiota interactions are important to maintain a state of immune tolerance to limit inappropriate responses in the gut[40,41]. When this homeostasis is perturbed, as in the context of infection-induced intestinal inflammation, this can lead to a marked dysbiosis and alterations in gut permeability and bacterial translocation that trigger immune-mediated tissue damage[42]. Depletion of the microbiota before infection with *Cryptosporidium* results in higher parasite burden[25,43]. This observation could indicate that the microbiota competes for resources required for parasite growth or that it provides tonic signals to the local immune cell populations or IECs that make the host intestine a less hospitable environment for *Cryptosporidium*.

The first physical barrier encountered by *Cryptosporidium* and other intestinal microorganisms is the mucus layer (Fig. 2). This barrier interfaces with the immune system, as glycans associated with mucin proteins have anti-inflammatory effects on dendritic cells[44]. To breach the mucus layer, microorganisms utilize various strategies, which include active motility or the production of mucolytic enzymes[45]. Pathogens may also target regions where the mucus layer is reduced, such as the dome epithelium above Peyer's patches, which lacks mucus-secreting goblet cells[45]. Here, the paucity of mucus permits microfold cells to sample the microbiota but can potentiate infection[45]. Although *Cryptosporidium* has been observed in microfold cells[46], it does not appear that the parasite preferentially targets these cells, and how *Cryptosporidium* can bypass the mucus layer and access the intestinal epithelium remains poorly understood.

Once the mucus layer is breached, pathogens reach the epithelial barrier, which predominantly comprises enterocytes specialized for nutrient uptake[47]. From the perspective of the parasite, this provides a large, dense surface area of homogenous cell types suitable for infection. However, there are also IEC subpopulations that perform specialized functions, including goblet cells (mucus production), microfold cells (antigen transport), tuft cells (innate sensing) and Paneth cells (production of antimicrobial peptides (AMPs)). Of these, *Cryptosporidium* has been detected in microfold cells[46] and it is less clear whether there is infection of other rare IEC subtypes. Incubation of *Cryptosporidium* with AMPs produced by Paneth cells, such as defensins, the cathelicidin LL37 and lysozyme, can reduce sporozoite viability in vitro[48–50]. However, the role of endogenous AMPs during infection is unclear, and additional studies are needed to define the role of Paneth cells and other IEC subtypes in the pathogenesis of cryptosporidiosis.

Once established in the host, *Cryptosporidium* causes severe, watery diarrhoea by an incompletely understood mechanism. Three possibilities have been suggested: (1) parasite-induced malabsorption that leads to osmotic diarrhoea; (2), secretory diarrhoea that occurs due to parasite-induced inflammatory responses and production of neuropeptides, such as substance P; and (3), secretory diarrhoea caused by an as-yet-to-be identified *Cryptosporidium*-derived enterotoxin[29]. In mice, *C. parvum* infection is associated with impaired ion transport and $Na^+$–glucose absorption[51]. This may be due, at least in part, to rapid parasite growth accompanied by cell death, changes in tight and adherens junctions, and loss of the microvillar brush border and glycocalyx[2,26,29,52] (Fig. 2). Clinically, reduced absorption of vitamin $B_{12}$ and D-xylose, a sugar that is readily absorbed in healthy intestines, has been observed in a cohort of patients with concomitant AIDS and cryptosporidiosis, which also supported a mechanism of osmotic diarrhoea[53]. By contrast, in infected macaques, treatment of tissue samples with a substance P receptor antagonist restored proper ion secretion and glucose absorption[54]. In addition, the low faecal osmotic gap (a comparison of solute concentrations between serum and faeces) in paediatric patients with cryptosporidiosis suggested that the diarrhoea was secretory[55,56]. These observations imply that *Cryptosporidium*-induced diarrhoeal disease is multifactorial in nature.

## Box 1

# Effect of recent technologies on studies of the immune response to *Cryptosporidium*

Initial efforts to genetically modify *Cryptosporidium* were impeded by unique aspects of its biology. The oocyst is resistant to transfection and the ability to excyst and transduce short-lived sporozoites is dependent on their rapid use for oral infection[172]. *Cryptosporidium* species lack enzymatic machinery for non-homologous end joining, which means that CRISPR–Cas9 and other technical advances were critical for genetic modification. Parasites have been engineered to express fluorescent proteins[26,28,31] and nanoluciferase[173], which permits fast and rigorous quantification of parasites in culture or in vivo. This latter approach provides a powerful tool to assay infection kinetics when the parasite[174] or the host immune system[25,28,175] is modified. *Cryptosporidium* transgenesis also permits the expression of model antigens in parasites[176]. This approach, combined with congenically marked TCR transgenic T cells that can be easily identified in vivo, has been used to study T cell responses to an array of bacteria and parasites[177–180].

One limitation of transformed cell lines is an inability to support *Cryptosporidium* fertilization and oocyst release[37]. More recent work has taken advantage of enteroid cultures, which can support asexual and sexual stages of *Cryptosporidium parvum* replication and from which oocysts can be recovered[164,165]. A variation of this approach is air–liquid interface monolayers, in which enteroids are disrupted, seeded onto a transwell and cultured for several days before media are removed from the upper chamber[164]. This approach provides the ability to infect the apical side of the polarized epithelial cell monolayer and analyse the impact of adding drugs, immune cells and cytokines to the basolateral side. A related strategy applied to *Cryptosporidium* is the use of a microchannel gel scaffold that mimics the crypts and villi of the small intestine and offers a coverslip 'window' into the device and ports to introduce cytokines and other factors into the system[181].

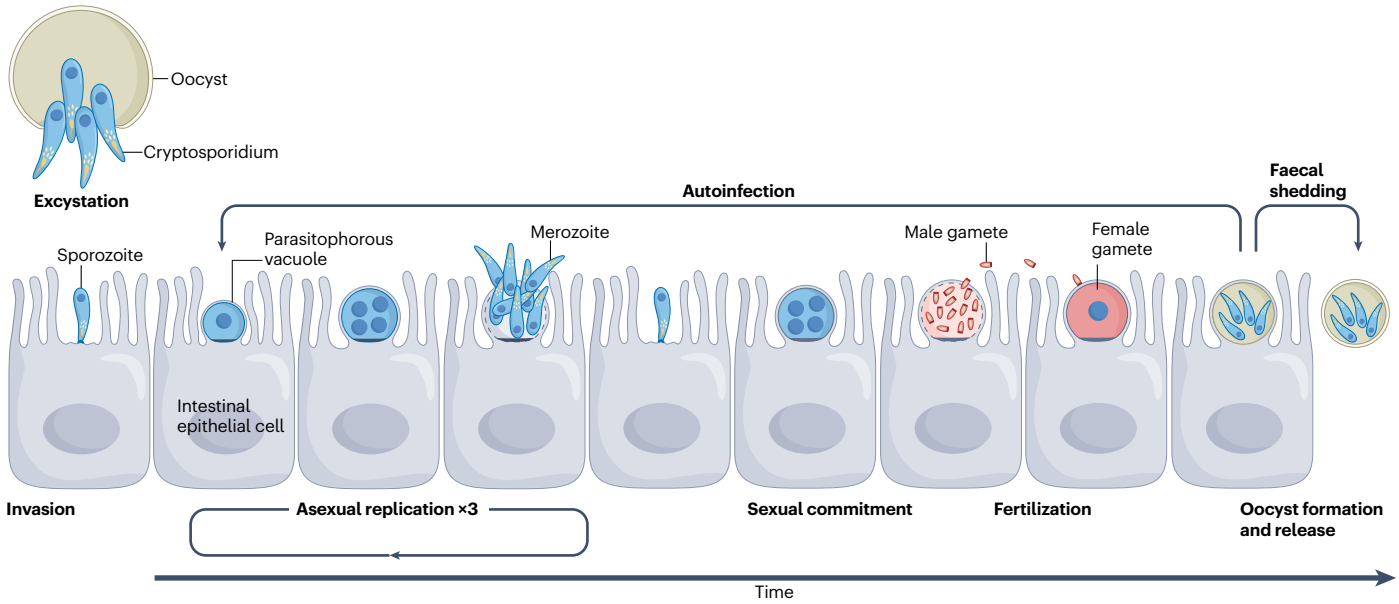


**Fig. 1 | *Cryptosporidium* life cycle.** Following oocyst ingestion and excystation, motile sporozoites infect intestinal epithelial cells, where they reside in an extracytoplasmic, but intracellular, parasitophorous vacuole at the apical edge of the cell. Asexual replication generates eight merozoites, which egress and infect neighbouring intestinal epithelial cells. This cycle occurs three times, after which merozoites undergo a sexual fate decision, leading to either 16 male gametes or a single female gamete. Males fertilize the intracellular female leading to production of oocysts, which continue the life cycle of the parasite either via faecal shedding and infection of a new host or autoinfection of the current host.

However, even in children that do not have overt symptoms, *Cryptosporidium* infection often has a profound and long-lasting effect on nutrient and ion absorption that contributes to chronic malnutrition[10].

A key feature of the digestive tract is the presence of spatially polarized processes to renew the IEC barrier. Thus, intestinal stem cells in the crypts generate new IECs that mature and differentiate as they progress up the villus before being shed at the tip[57,58]. This orderly IEC escalator ensures barrier maintenance and is important for repair but is modulated in response to enteric infection and cellular stresses. For many infections, including *Cryptosporidium*, this is manifested as stem cell hyperplasia and crypt branching, increased turnover of IECs and villus lengthening[26,59–61]. Increased cell death during infection can also lead to pathological changes that include villus atrophy, which is a prominent feature associated with cryptosporidiosis[29] (Fig. 2). Although increased cell turnover generates new targets for infection, IEC extrusion at the villus tip means that cells nearest the tip will have a short lifespan, which could preclude the ability of *Cryptosporidium* to complete its intracellular cycle. Indeed, extrusion of infected IECs is considered an evolutionarily conserved mechanism of resistance that limits pathogen expansion by premature loss of their host cell niche[60,62,63]. It seems likely that this rapid turnover of enterocytes has shaped the evolution of *Cryptosporidium* and is responsible for its rapid intracellular development. By contrast, other intestinal pathogens may benefit from this process. For example, extrusion of IECs infected by *Salmonella enterica* subsp. *enterica* serovar Typhimurium leads to the release of cytosolic reservoirs of the bacteria and enhanced dissemination within the host[64]. How these processes of cell death and IEC shedding impact *Cryptosporidium* infection, and whether *Cryptosporidium* exploits these processes for oocyst release, are important topics for future study.

## Innate sensing of *Cryptosporidium*

One of the major principles that underlies resistance to infection is that innate sensing of pathogens or pathogen-mediated damage provides signals that initiate and amplify a coordinated immune response. The range of Toll-like receptors (TLRs) expressed by humans (TLR1–TLR10) and mice (TLR1–TLR9 and TLR11–TLR13) enables detection of distinct pathogen-associated molecular patterns that include virally derived double-stranded RNA (sensed by TLR3), bacterially derived lipopolysaccharide (detected by TLR4) and teichoic acid synthesized by Gram-positive bacteria (detected by TLR2)[65]. The detection of a pathogen-associated molecular pattern by a TLR activates NF-κB and MAPK signalling, which are conserved pathways associated with innate immunity[65]. These events typically result in transcriptional responses characterized by the production of cytokines and chemokines and promote antimicrobial activities that underlie resistance to infection[65].

IECs express a broad array of pattern recognition receptors (PRRs) and are well equipped to be the initial sensors of *Cryptosporidium* infection. The localization of the membrane-associated TLRs inside endosomes or on the basal surface should restrict interactions with luminal microorganisms unless they invade the cell or breach the barrier[66]. Although *Cryptosporidium* is intracellular, its sequestration in a parasitophorous vacuole at the apical surface of the IEC may shield this organism from some innate sensors. Nevertheless, in vitro exposure of IECs to *Cryptosporidium* can promote the production of cytokines (type I and type III interferons and IL-18)[27,67–71] that mediate innate resistance to *Cryptosporidium* (Fig. 3) and other intracellular pathogens. In addition, several studies using transformed IEC-derived cell lines have implicated TLRs in the response to *Cryptosporidium*. Exposure to live (but not heat-killed) *C. parvum* resulted in TLR2-dependent and TLR4-dependent NF-κB activation[72,73].

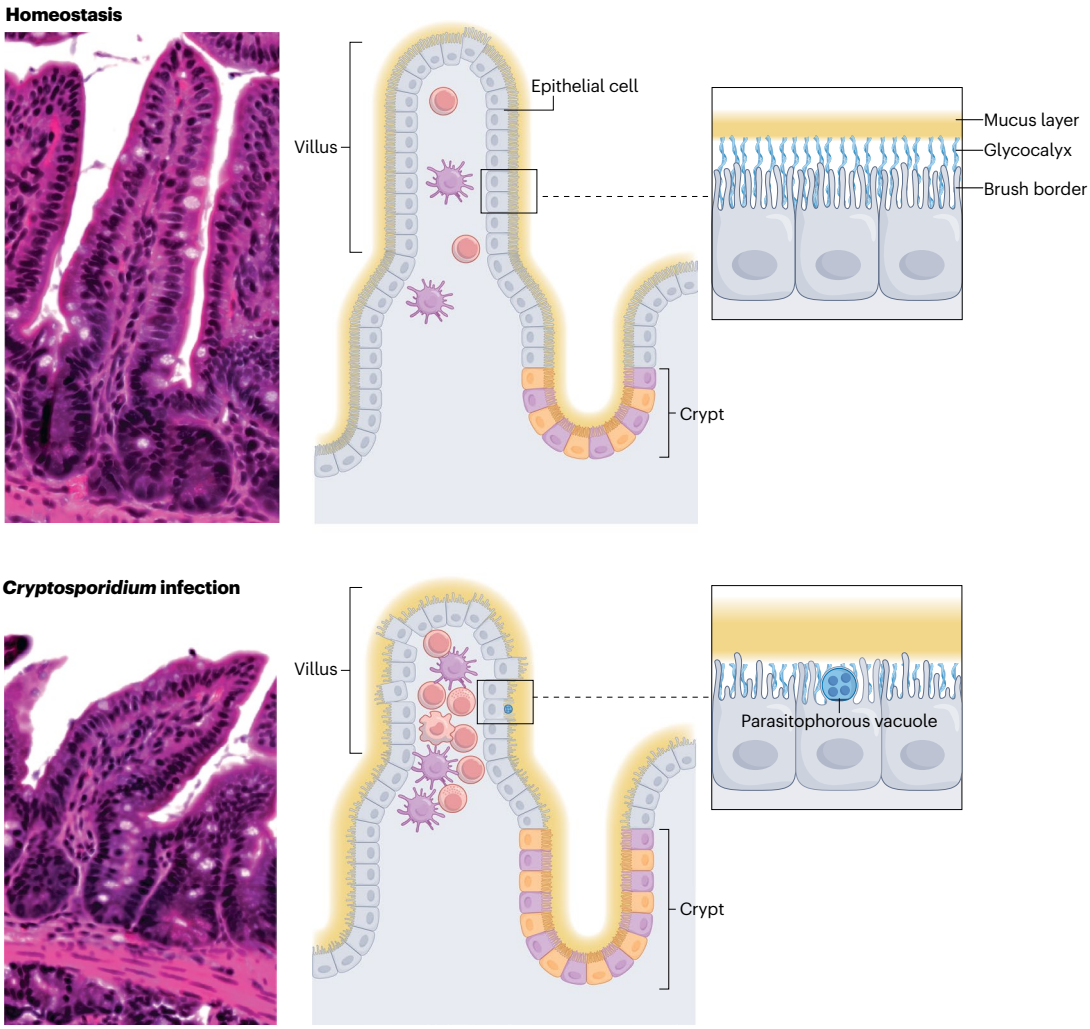


**Fig. 2 | Pathological changes during *Cryptosporidium* infection.** At homeostasis, new intestinal epithelial cells are generated in the crypts, which maintain the epithelial barrier as older intestinal epithelial cells are shed into the lumen at the villus tip. At a microscopic level, the epithelial barrier consists of a mucus layer and glycocalyx to ensure host–microbiome separation and a microvillar brush border that contributes to nutrient absorption. During infection, increased intestinal stem cell turnover leads to crypt deepening; however, damage and cell death cause villus blunting and epithelial hyperplasia along the villi. This can be seen in representative haematoxylin and eosin-stained sections of an uninfected villus (top left) compared with a villus from a *Cryptosporidium*-infected mouse (bottom left) (original magnification, ×20). Infiltration of immune cells into the vessels within the villus can also be seen. Microscopic changes include loss of the glycocalyx and brush border, which reduces the capacity of the host to maintain a barrier to microorganisms and impacts nutrient and ion absorption, probably contributing to diarrhoeal disease associated with infection.

Consistent with these observations, TLR4 contributes to parasite control in a mouse model of biliary cryptosporidiosis[74]. Furthermore, treatment of neonatal mice with the TLR3 agonist polyinosinic:polycytidylic acid (a synthetic double-stranded RNA analogue) reduced parasite burden[75]. This result foreshadowed the finding that TLR3 is required for *C. parvum*-induced production of IFNλ (a member of the type III interferon family)[27,68] (Fig. 3). It is noteworthy that neonatal mice have reduced levels of TLR3 in the small intestine, which is associated with heightened susceptibility to rotavirus infection[76]. Whether this helps to explain the increased susceptibility of neonatal mice[23] or children to *Cryptosporidium* compared with adults is unclear.

The NOD-like receptors (NLRs) are a family of more than 20 proteins that are specialized to detect perturbations to the cell cytosol, which can include the presence of microorganisms. The defining feature of NLRs is a modular organization of distinct domains involved in pathogen-associated molecular pattern detection and the formation of multimeric complexes (the inflammasome) that activate innate signalling pathways and, in some instances, cell death. Certain members of the NLR family, such as NLRP3 (also known as NALP3), form inflammasomes that activate caspase 1, which leads to proteolytic processing and activation of IL-1β and IL-18 (ref. 77). IL-18, a member of the IL-1 family of cytokines, is required for innate resistance to *Cryptosporidium*[25,78,79], and exposure to *C. parvum* leads to IL-18 release in vitro[67,80,81]. Although three NLR family members (NLRP1b, NLRP3 and AIM2) have been implicated in resistance to other apicomplexans[82,83], they are not required for control of *C. parvum*[25]. By contrast, caspase 1

and NLRP6 (also known as NALP6) are required for release of IL-18 and optimal control of *C. parvum*[25,67] (Fig. 3). NLRP6 also contributes to detection of other enteric pathogens, including murine norovirus and *C. rodentium*[84,85]. NLRC4 inflammasome activation and IL-18 release restrict *S.* Typhimurium infection; although in this system, evidence exists that an initial cytokine signal is required to 'prime' IECs for optimal inflammasome activation[60,86]. The conserved role of inflammasomes, in particular NLRP6, as important initiators of host immunity in the small intestine is complicated by studies that have linked this sensor to microbiome-derived signals[85]. Regardless, optimal immunity to *Cryptosporidium* requires the NLRP6 inflammasome in both the presence and the absence of the microbiome[25].

With the identification of TLR3 and NLRP6 in mice as sensors of *Cryptosporidium*, it is relevant to consider what features of this infection lead to the activation of these PRRs. The observation that live parasites are required for TLR and inflammasome activation implies that these responses are dependent on pathogen-derived factors or metabolites that may access the cell cytosol or endosomal system. The identification of *Cryptosporidium* proteins that are translocated into the infected cell[31,87] highlights that, despite its unusual intracellular but extracytoplasmic location, this organism can modify its host cell. One of these translocated factors targets LIM domain-only protein 7 (LMO7), a component of the actin cytoskeleton[31]. Although manipulation of the actin cytoskeleton by other microorganisms contributes to cellular invasion or cell-to-cell spread, pathogen-induced changes in the actin machinery can be sensed by the host and trigger inflammasome activation[88,89]. Whether the events that lead to the formation of the actin pedestal associated with *Cryptosporidium* contribute to innate recognition remains to be tested.

It has been reported that *Cryptosporidium*-derived RNA is trafficked to the nucleus of infected host cells[90], which could trigger TLR3. *Cryptosporidium* also contains *Cryspovirus*, a double-stranded RNA viral symbiont[91], and *Cryspovirus* RNA in the cytoplasm of infected cells can activate protein kinase R (PKR) and retinoic acid-inducible gene I (RIG-I)-dependent type I interferon production[71]. Although knockdown of TLR3 had no effect on type I interferon stimulation[71], whether *Cryspovirus* can activate NLRP6 remains to be tested. The gut also contains an array of dendritic cell subtypes (discussed below), which express many of the same innate sensors as IECs. These cells are not infected by *Cryptosporidium*, but activated dendritic cells exposed in vitro to *C. parvum* produce several cytokines including IL-12 (refs. 81,92). Moreover, in mice infected with *Cryptosporidium*, there is increased dendritic cell production of IL-12p40 (a subunit shared between the cytokines IL-12 and IL-23) in the small intestine[24]. How these specialized immune cells access and sense *Cryptosporidium* remains an open question that is discussed in more detail below.

## Innate resistance to *Cryptosporidium*

Viral, bacterial, protozoan and helminth pathogens utilize diverse mechanisms to establish infection in the small intestine. This is reflected in the multiple forms of innate immunity in the gut that prevent such establishment or provide short-lived mechanisms of resistance before the development of adaptive responses. Early type I and type III interferon derived from *Cryptosporidium*-infected IECs contribute to parasite control within IECs[27,68,70], although type I interferon has also been suggested to limit host resistance[27,71]. Beyond these IEC-intrinsic pathways, additional mechanisms detect infection and amplify the cellular response to create a local environment that is hostile to the pathogen (Fig. 4). For many intracellular pathogens, including *Cryptosporidium*, this is exemplified by pathways that lead to the induction of IFNγ (produced by various lymphocyte populations), which activates mechanisms to restrict parasite growth[28,93].

Mice deficient for IL-12p40 or IL-12p35 (which is unique to IL-12) are highly susceptible to *C. parvum*, and IL-12 treatment promotes parasite control[78], consistent with a dominant role for IL-12 in resistance to this infection. IL-18 released by infected IECs stimulates IFNγ production by innate immune cells, although during *S.* Typhimurium and rotavirus infections,

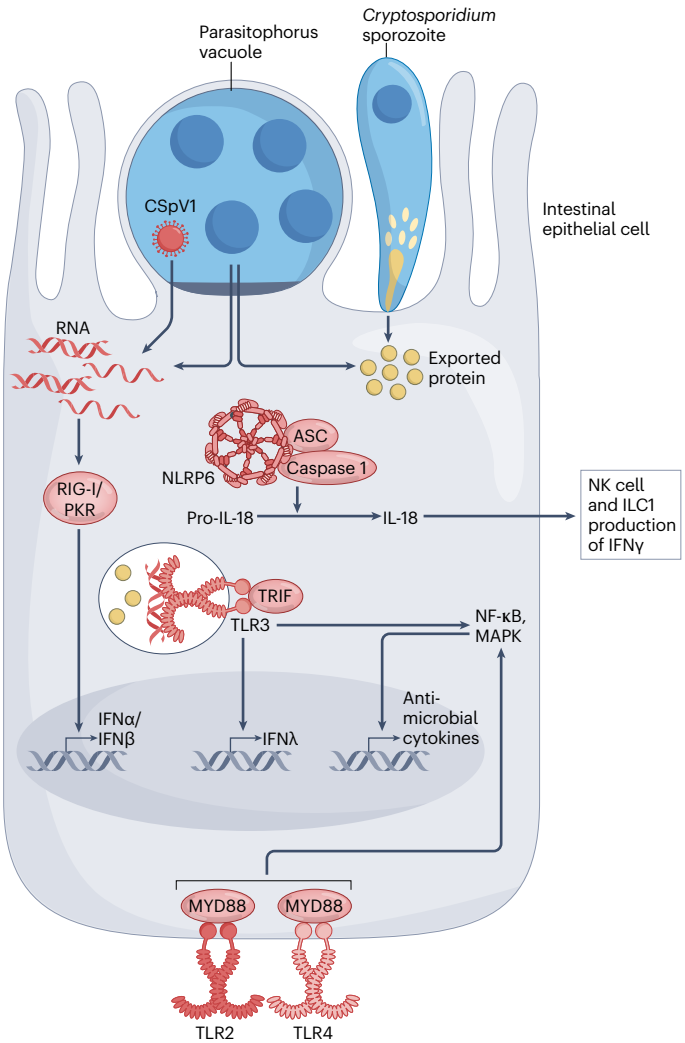


**Fig. 3 | Sensing of infection by intestinal epithelial cells.** Infection leads to NLRP6 inflammasome activation, which mediates the conversion of pro-IL-18 to its active form, which is secreted and stimulates IFNγ production by immune cells such as natural killer (NK) cells and type 1 innate lymphoid cells (ILC1s). In addition, activation of Toll-like receptor 3 (TLR3) by *Cryptosporidium* leads to the production of IFNλ to limit parasite replication in neighbouring cells. Additional TLRs such as TLR2 and TLR4 have been implicated in *Cryptosporidium* detection, which may contribute to host defence in epithelial cells in the biliary tree. Although the precise pathogen-derived signals are not well understood, *Cryspovirus* (CSpV1) double-stranded RNA is detected by retinoic acid-inducible gene I protein (RIG-I) and protein kinase R (PKR) in the host cytoplasm, leading to type I interferon production. Additional candidate ligands include parasite proteins translocated into the host cell and reports of *Cryptosporidium* RNA being translocated to the host cell nucleus.

this cytokine can promote AMP production and killing of infected cells[63,94]. Initial studies using severe combined immunodeficiency (SCID) mice, which lack T cells and B cells, identified natural killer cells as an innate source of IFNγ that contributed to resistance to *Cryptosporidium*[12,21,95]. It is now recognized that there are additional tissue-resident populations of innate lymphoid cells (ILCs)—ILC1, ILC2 and ILC3—that are associated with specific responses to different enteric challenges[96]. This has led to a model in which dendritic cell production of IL-12 acts in synergy with IL-18 to induce natural killer cell and ILC1 production of IFNγ that promotes control of *Cryptosporidium* infection[28] (Fig. 4).

Although the ability of natural killer cells and ILC1s to produce IFNγ remains the best-characterized mechanism of innate resistance to *Cryptosporidium*, there are studies that indicate that other ILC populations may contribute to parasite control. Stimulation of ILC3 with IL-23 (a cytokine composed of the shared IL-12p40 subunit and unique p19 subunit) results in the production of IL-17 and IL-22; both directly impact IECs, as IL-17 promotes tight junction formation and barrier function[97], whereas IL-22 induces AMP production[98] and contributes to control of rotavirus, *Eimeria* and *C. rodentium* infections[63,99,100]. The finding that mice deficient for IL-12p40 treated with IL-23 have lower *Cryptosporidium* burdens[78] may be due to the capacity of IL-23 to promote IFNγ production (although less potently than IL-12)[101] or to activate ILC3s. Interpretation of these data is limited by the lack of studies that address the role of IL-17 or IL-22 in resistance to *Cryptosporidium*. Nevertheless, it seems likely that optimal parasite control may require complementary contributions by ILC1s and ILC3s that have an effect on different elements of intestinal and immune biology.

## Induction of T cell responses to *Cryptosporidium*

The clinical observation that primary or acquired defects in T cell function are associated with increased susceptibility to *Cryptosporidium* established the importance of T cells in the control of this organism. In individuals infected with HIV, the use of highly active antiretroviral therapy, which restores mucosal and circulatory $CD4^+$ T cell numbers, results in resolution of *Cryptosporidium* infection[102]. Understanding the role of T cells in parasite control is complex as there are many different T cell populations present in the gut. Conventional αβ-$CD8^+$ and $CD4^+$ T cells that recognize peptide antigens presented in the context of MHC class I (MHC-I) and MHC-II molecules, respectively, form the basis for pathogen-specific responses. Conversely, $FOXP3^+CD4^+$ regulatory T cells have important roles in local tolerance and mitigate the pathological effects of inflammation in the gut[103]. There are also unconventional T cell receptor-$\alpha\beta^+$ ($TCR\alpha\beta^+$) and $TCR\gamma\delta^+$ T cells that express CD8αα homodimers or are negative for CD4 and CD8 (ref. 104). Many of these intraepithelial lymphocytes have a phenotype consistent with previous TCR activation and their ability to produce TGFβ contributes to maintenance of the epithelial barrier[104]. At homeostasis, subsets of these cells appear to readily express IFNγ and IL-17 (ref. 104), which makes it difficult to distinguish cells that are specific for *Cryptosporidium* versus those that are responsive to microbiota-derived antigens. Although an endogenous MHC-I-restricted *Cryptosporidium* epitope has recently been described[105], advances in parasite transgenesis and the opportunity to introduce model antigens into *Cryptosporidium* may help to distinguish T cell responses to *Cryptosporidium* (Box 1).

On the basis of studies from other experimental systems, it seems likely that dendritic cells acquire *Cryptosporidium* antigen in the gut and traffic to draining lymph nodes (or the T cell area of Peyer's patches) to prime naive T cells. This would result in expansion, differentiation and homing of parasite-specific effector T cells back to the gut to mediate local control (Fig. 5). Following priming, activated $CD4^+$ T cells can develop into distinct T helper subsets ($T_H1$, $T_H2$ and $T_H17$) that are associated with resistance to different classes of pathogens. For many intracellular pathogens, including *Cryptosporidium*, dendritic cell-derived IL-12 promotes a $T_H1$-like response, dominated by production of IFNγ, which is critical for resistance to infection. Indeed, global depletion of dendritic cell populations in neonatal mice leads

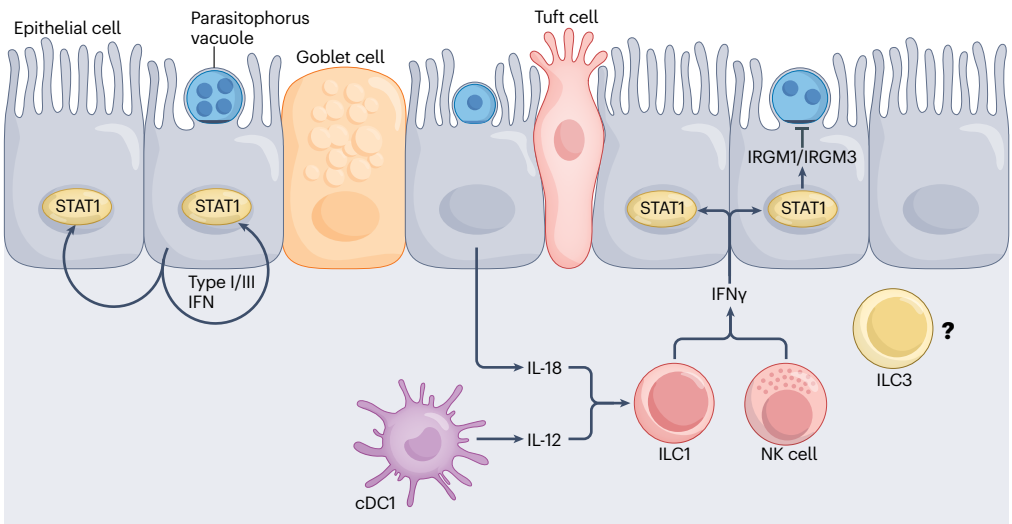


**Fig. 4 | Innate responses to *Cryptosporidium* infection.** Autocrine and paracrine signalling of type I and type III interferons (IFNs) to epithelial cells leads to STAT1-dependent activation of cell-intrinsic mechanisms of parasite control. In addition, release of IL-18 by infected epithelial cells and IL-12 produced by gut-resident dendritic cells (DCs) stimulates type 1 innate lymphoid cells (ILC1s) and natural killer (NK) cells to produce IFNγ. This IFNγ production activates STAT1 in epithelial cells, which leads to expression of IRGM1 and IRGM3 that limit parasite replication. Although ILC3s and related cytokines such as IL-23 may contribute to parasite control, a direct role has not been elucidated (indicated by '?'). Similarly, roles for additional epithelial cell subpopulations such as goblet cells (which specialize in mucus secretion) and tuft cells (which are involved in innate sensing) have yet to be described. cDC1, conventional dendritic cell 1.

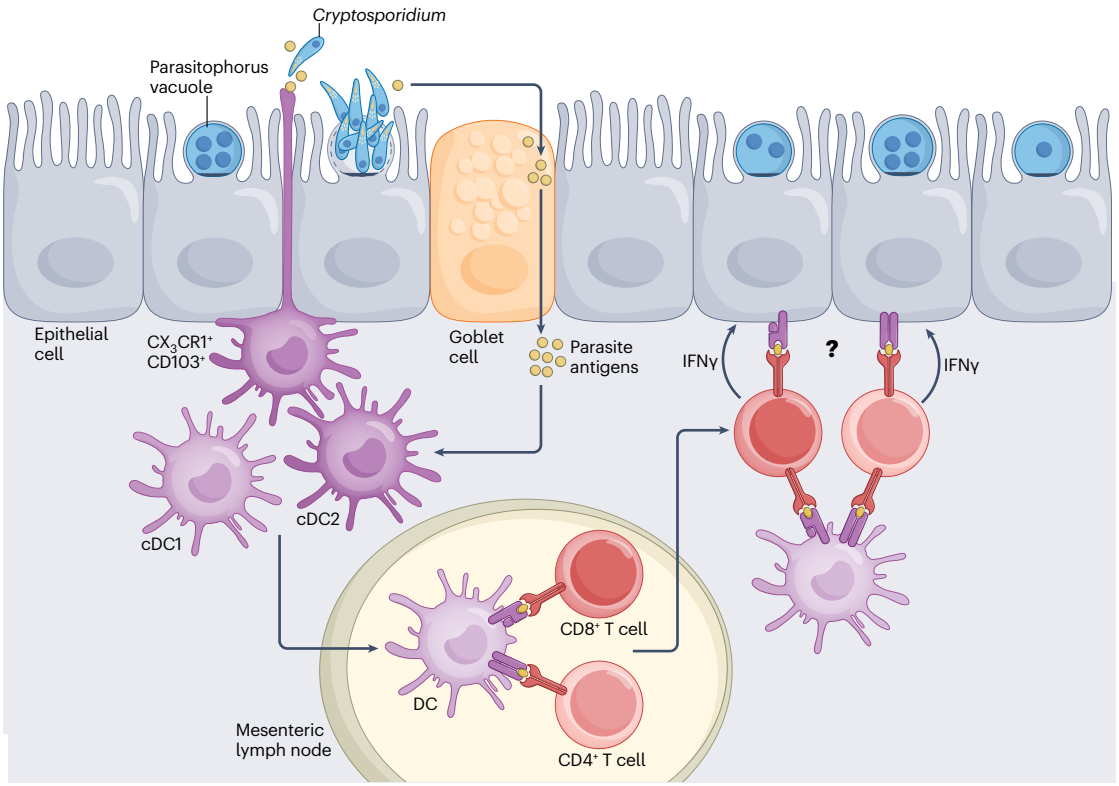


**Fig. 5 | Adaptive responses to *Cryptosporidium* infection.** As parasite and immune cells are spatially separated by intestinal epithelial cells (IECs), questions remain as to how the host acquires antigen for induction of T cell-mediated immunity. One method may be via gut-resident CD103$^+$ conventional dendritic cells (cDCs) and $CX_3CR1^+$ macrophages that extend processes between IECs to sample antigen in the intestinal lumen. Alternatively, goblet cells have been described to act as antigen channels, transporting intestinal antigen to cDCs in the lamina propria. Antigen may also be acquired from the uptake of debris from the death of infected cells. Following antigen acquisition, cDCs traffic to the mesenteric lymph nodes and present antigen to activate naive CD4$^+$ and CD8$^+$ T cells. After expansion of antigen-specific T cell populations and their trafficking to the gut, the production of IFNγ by these activated T cells acts on the infected IEC to restrict infection. Whether IECs can directly present antigen to T cells and the contributions of antigen presentation and cytokine production by gut-resident cDCs to T cell-mediated immunity remain to be defined (indicated by '?').

to increased susceptibility to *Cryptosporidium* infection, associated with reduced levels of mRNA encoding IL-12p40 and IFNγ[106]. However, the small intestine contains multiple dendritic cell subsets that have distinct properties that could be involved in the generation of parasite-specific T cells. For example, conventional dendritic cells 1 (cDC1s) are a major source of IL-12 and have an enhanced capacity to sample the local environment and to cross-present endogenous antigens to CD8$^+$ T cells[107]. Mice that lack cDC1s are more susceptible to *Cryptosporidium*, which has been attributed to a reduced capacity to produce IL-12 and IFNγ[24,108]. Conversely, cDC2s are considered specialized to process exogenous antigens and preferentially prime CD4$^+$ T cells[107]. It is important to note that these conventions are best considered as a general guideline as both cDC subsets express MHC-I and MHC-II and are capable of priming CD8$^+$ and CD4$^+$ T cells[109].

In neonatal and adult mice infected with *C. parvum*, dendritic cells are recruited to the ileum[110], and *C. parvum* antigen has been detected in activated dendritic cells in the gut-draining mesenteric lymph nodes[92]. Macrophages are also recruited to the lamina propria during *C. parvum* infection[111] and early experiments that used electron microscopy have identified intact and partially degraded parasites inside mononuclear phagocytic cells in Peyer's patches[46]. A protective role for these cells has been suggested by experiments in which depletion of macrophages from *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice or macrophages and neutrophils from SCID beige mice increased *Cryptosporidium* burden[79,95]. As these cells are not infected by *Cryptosporidium* and their location in the lamina propria is spatially separated from the parasite, fundamental questions remain about how these cells acquire antigen from a parasite that dwells in the epithelium and the lumen of the gut. There are specialized populations, including CD103$^+$ dendritic cells and $CX_3CR1^+$ macrophages, that can extend processes through the IEC layer into the lumen of the small intestine for antigen sampling[112,113] (Fig. 5). Another possibility includes the ability of goblet cells to sample antigen and 'pass' them to antigen-presenting cells in the lamina propria[114] (Fig. 5). Furthermore, the death of infected IECs may provide the opportunity for local antigen-presenting cells to acquire parasite antigen, and studies in a reductionist model system have demonstrated that inflammasome activation in IECs contributes to the ability of dendritic cells to acquire antigen and prime CD8$^+$ T cells[115]. As IEC expression of NLRP6 is important for detection of *Cryptosporidium*[25], it is possible that a similar mechanism of IEC-intrinsic inflammasome activation (and perhaps cell death) could contribute to dendritic cell activation and induction of T cell-mediated immunity during cryptosporidiosis. There are probably

multiple mechanisms that allow dendritic cells to sample antigens and comparisons with other pathogens can provide the opportunity to identify conserved pathways that are tailored to different classes of enteric immune responses. This in turn may yield information for the design of new vaccination strategies that induce mucosal T cell responses crucial for parasite control.

## T cell-mediated resistance to *Cryptosporidium*

Numerous studies have established that both $CD4^+$ and $CD8^+$ T cells contribute to clearance of *Cryptosporidium*[116–118], but following priming, how these cells re-encounter parasite-derived antigens at the site of infection is uncertain (Fig. 5). The ability of local dendritic cells in the lamina propria to sample and present antigen in the context of MHC-I and MHC-II may lead to microenvironments rich in IFNγ or other T cell-derived effectors that act on IECs to limit parasite replication. Alternatively, IEC expression of MHC-I and MHC-II raises the possibility that T cells respond directly to infected IECs. A recent report has also described antigen-independent, IL-12-dependent and IL-18-dependent activation of IFNγ production by $CD4^+$ T cells recruited to the small intestine during murine norovirus or murine adenovirus 2 infection[119]. Thus, the theme of how T cells are activated locally in the IEC compartment is broadly relevant to diverse enteric infections.

Although T cells and IFNγ are both required for sterile immunity to *Cryptosporidium*, multiple studies have highlighted a $CD4^+$ T cell-dependent, IFNγ-independent pathway of parasite control[26,116]. Analysis of additional $T_H$ cell subsets induced during infection with *C. parvum* and *C. tyzzeri* has revealed low numbers of parasite-specific, IL-$4^+$ $T_H2$ cells[24,120]. As the development of $T_H2$ cells is antagonized by IL-12 and IFNγ, this subset was not expected to be associated with *Cryptosporidium* infection. However, and surprisingly, the absence of IL-4 is associated with delayed parasite clearance[120]. One possible explanation is the effect of IL-4 on intestinal physiology and motility that is required for expulsion of helminths[121] may also contribute to resistance to *Cryptosporidium*. Indeed, patients with Hirschsprung disease, a rare genetic disorder that affects intestinal motility, are more susceptible to *Cryptosporidium* infection[122]. Alternatively, it is possible that these cells mediate resistance independently of canonical $T_H2$-associated activities. The CD40L molecule (a member of the TNF superfamily) is important for resistance to *Cryptosporidium* in humans and mice[123,124] and is expressed by activated $CD4^+$ cells. The interaction of CD40L with CD40 on dendritic cells has been linked to the induction of IL-12 and to a process termed 'licensing' that enables dendritic cells to support optimal $CD8^+$ T cell activation[125–127]. Furthermore, CD40L can interact with CD40 expressed by haematopoietic and non-haematopoietic cells to limit the replication of the related parasite *Toxoplasma gondii*[128]. There are several readily available approaches to study the biology of CD40–CD40L interactions, which include the capacity to identify cells that interact via these molecules using Labelling Immune Partnerships by SorTagging Intercellular Contacts (LIPSTIC)[129]. Such tools provide an opportunity to understand how this pathway functions to limit *Cryptosporidium* infection and how this intersects with IFNγ-mediated immunity.

As noted earlier, the cytokine IL-23 is related to IL-12 but promotes the development of $T_H17$ cell responses, characterized by $CD4^+$ T cell production of IL-17 and IL-22. Several studies have described increased levels of IL-17 during *Cryptosporidium* infection[130–132], and treatment of mice deficient for IL-12p40 with IL-23 is sufficient to reduce parasite burden[78]. This latter observation may infer a protective effect of IL-23-driven ILC3 or $T_H17$ cell responses during *Cryptosporidium* infection. This class of immune response is most prominently associated with resistance to certain fungal organisms and, in the small intestine, the Gram-negative bacterium *C. rodentium*[98,133]. This immunobiology is further complicated by the observations that high levels of IL-23 can promote pathological $T_H17$ cell responses in the gut[134], but $T_H17$ cells also contribute to maintenance of the mucosal barrier. For example, IL-17 promotes tight junction formation[97] and IL-22 can enhance intestinal stem cell proliferation and the IEC escalator[63]. As discussed above, the host response to *Cryptosporidium* is associated with disruption of the epithelial barrier, including IEC death, crypt hyperplasia, increased turnover of IECs and villus blunting. The $T_H1$ and $T_H17$ cell responses can intersect with these processes and, during a helminth infection, IFNγ has been shown to drive a 'fetal-like reversion' of the intestinal stem cell niche associated with an increased proliferative capacity to facilitate barrier repair[61]. However, whether $T_H17$ cell-derived cytokines influence these processes independently of IFNγ and contribute to *Cryptosporidium* control or resolution of tissue damage is unclear.

The ability of MHC-I-restricted $CD8^+$ T cells to directly recognize and kill infected cells, as well as produce IFNγ and TNF, underlies their role in resistance to many intracellular infections[135]. Experimental studies using *C. parvum* have described $CD8^+$ T cell accumulation and IFNγ production in the intraepithelial lymphocyte compartment[136,137], and $CD8^+$ T cells from human patients previously infected with *Cryptosporidium* can lyse infected cells[138]. However, studies in mouse models have shown that mice deficient for MHC-I or depleted of $CD8^+$ T cells shed similar amounts of oocyst to control mice, whereas MHC-II-deficient and $CD4^+$ T cell-depleted mice are more susceptible to *Cryptosporidium*[116,117]. By contrast, depletion of both $CD4^+$ and $CD8^+$ T cells promotes higher susceptibility than depletion of $CD4^+$ T cells alone[116], and adoptive transfer of total $CD4^+$ and $CD8^+$ T cells from *Cryptosporidium*-immune mice protects naive mice better than transfer of $CD4^+$ T cells alone[139]. One interpretation of these results is that the $CD4^+$ T cell response is dominant, and only in its absence does the contribution of $CD8^+$ T cells become apparent. Similar findings have been reported for the enteric pathogens *C. rodentium* and *S.* Typhimurium, where the $CD4^+$ T cell response mediates resistance and, in the case of *C. rodentium*, the $CD8^+$ T cell response is considered dispensable[140,141]. This interpretation is complicated by the recognition that $CD4^+$ T cells

## Glossary

**Merogony**
A form of asexual replication by which the parasite nucleus divides multiple times followed by segmentation into eight daughter parasites.

**Micronemes**
Secretory organelles located in the apical third of apicomplexan parasites that are associated with parasite motility and cell invasion.

**Nitazoxanide**
The only drug approved for the treatment of *Cryptosporidium* in otherwise healthy adults and children.

**Parasitophorous vacuole**
A cell compartment derived during parasite invasion from the host plasma membrane. Here, the parasite completes its replication cycle, shielded from aspects of intracellular immunity.

**Rhoptry**
A club-shaped secretory organelle that apicomplexan parasites discharge into the host cell to initiate invasion and to deliver effector proteins into the infected cell.

have regulatory activities that include the ability to license dendritic cells that are critical to support $CD8^+$ T cell expansion[142]. As such, the absence of $CD4^+$ T cells (experimentally or clinically) could result in reduced $CD8^+$ T cell responses and thus contribute to the prominent role of $CD4^+$ T cells in resistance to this infection.

## Mucosal antibody responses to *Cryptosporidium*

An additional T cell subset induced following infection is T follicular helper ($T_{FH}$) cells, which interact with B cells that present cognate antigen[143] and promote germinal centre reactions and B cell affinity maturation. This process is mediated in part through $T_{FH}$ cell expression of CD40L, which interacts with CD40 on B cells[143] and induces antibody class-switching from IgM to other isotypes, which, at mucosal sites, are dominated by IgA and IgG[144]. Although $T_{FH}$ cells accumulate in the mesenteric lymph nodes and are crucial for protective IgA and IgG responses to *C. rodentium*[145], they have not been studied in the context of *Cryptosporidium*. It is important to note that although patients with hyper-IgM syndromes are characterized by the inability of B cells to undergo class-switch recombination, only those patients with mutations that specifically impact CD40L and CD40 show increased rates of *Cryptosporidium* infection[18]. This clinical observation may suggest a limited role for B cells in resistance to *Cryptosporidium* and is consistent with experimental infections in mice, which show that B cells are dispensable for resistance to primary infections with *C. parvum*[146] and *C. tyzzeri*[26]. Nevertheless, experimental infections of mice or humans with *Cryptosporidium* induces IgG and IgA antibodies, which can correlate with the magnitude of oocyst shedding in humans[147–150]. Studies in children have found that patients experiencing acute cryptosporidiosis had higher *Cryptosporidium*-specific IgA titres than patients with persistent diarrhoea[151,152]. Furthermore, a recent study that analysed the breadth and protective impact of *Cryptosporidium*-specific antibodies in a cohort of Bangladeshi children has found that antibody responses to the *Cryptosporidium* antigens Cp23 and gp60 correlated with protection against reinfection[153]. Thus, mucosal antibody responses may limit disease severity or susceptibility to reinfection, although additional studies will be necessary to better understand antibody function during *Cryptosporidium* infection and subsequent exposures.

## Epithelial cell-intrinsic control of *Cryptosporidium*

With the discovery of the important role of IFNγ in resistance to intracellular bacteria and protozoa came an early focus on its ability to enhance the antimicrobial activities of macrophages[154]. It is now recognized that IFNγ has much broader regulatory effects on the immune system and also induces distinct antimicrobial pathways in non-immune cells[93]. This is further complicated by the large variety of host species and cell-lineage-specific mechanisms downstream of IFNγ that limit the replication of different vacuolar pathogens. In vivo, the use of cell-type-specific deletion of STAT1, a transcription factor required for signalling by IFNγ (and other cytokines, including type I and type III interferons and IL-22), has helped to define which cell types are important for control of *Cryptosporidium*. This approach revealed that deletion of the gene encoding STAT1 in macrophages or dendritic cells does not result in increased susceptibility to *Cryptosporidium*, whereas loss of STAT1 in IECs causes a marked increase in susceptibility that is comparable with full-body deletion or depletion of IFNγ[28]. Likewise, the ability of exogenous IFNλ treatment to cause acute reductions in parasite burden is also dependent on IEC expression of STAT1 (ref. 27). These findings are consistent with a model in which interferons act directly on the IEC to promote parasite control (Fig. 4).

### Box 2

## How does *Cryptosporidium* evade the immune system?

A common theme of the study of host–pathogen interactions is the concept that the host possesses pathways that mediate resistance to infection, whereas pathogens develop strategies to subvert them. There are limited data on the molecular basis of *Cryptosporidium* immune evasion strategies, although cell cultures infected with *Cryptosporidium parvum* are resistant to apoptosis inducers such as staurosporine[182,183]. This observation suggests that *Cryptosporidium* may suppress host cell death pathways. In neonatal mice, *C. parvum* infection is associated with decreased expression of certain antimicrobial peptides (AMPs)[49,50,184], but how this is regulated is incompletely understood. Likewise, STAT1 signalling in enterocytes is critical to control *Cryptosporidium*, which decreases STAT1 expression in a mouse intestinal epithelial cell line[185].

Recent data have suggested that *Cryspovirus*-induced type I interferon production limits intestinal epithelial cell responsiveness to IFNγ[71], although both of these cytokines use STAT1 for signalling, highlighting a paradoxical role for type I interferon. Sensing by TLR3, NLRP6 and the production of interferons and IL-18 are important for parasite control and are therefore additional candidate pathways that may be targeted by *Cryptosporidium*. Many intracellular and extracellular pathogens deploy effectors into the host cell to initiate mechanisms of immune evasion. Proteins such as MEDLE2 (ref. 87) that are exported from *Cryptosporidium* are therefore obvious candidates for effectors that would subvert the host cell. A recent spatial proteomics screen has identified more than 150 additional parasite proteins secreted during infection[186], which suggests that *Cryptosporidium* probably targets multiple host pathways. Mechanisms of host immune evasion could underlie host specificity and relative virulence among the various species and strains of *Cryptosporidium*[187]. As *Cryptosporidium* follows a simple single-host sexual life cycle, it should be possible to conduct crosses between strains to map the genetic basis for varied host specificity or virulence.

Currently, the basis for how IFNs mediate restriction of *Cryptosporidium* is poorly defined, but the mechanisms of IFNγ-dependent control for other intracellular pathogens in non-haematopoietic cells may provide relevant insights. In one example, IFNγ limits intracellular iron availability that is required for the growth of some intracellular pathogens[93]. In cultures where IFNγ inhibited the growth of *Cryptosporidium*, the addition of exogenous iron ($FeSO_4$) antagonized this activity[155], although an important caveat is the ability of iron to induce internalization of the IFNγ receptor 2 chain[156]. Another well-characterized pathway to microbial restriction in non-haematopoietic cells involves the ability of IFNγ to induce indolamine dioxygenase (IDO), which catabolizes tryptophan and thereby deprives intracellular parasites of an essential amino acid[157]. However, in mice, IDO is dispensable for control of *Cryptosporidium*[28].

IFNγ also induces immune-related GTPases (IRGs) and guanylate-binding proteins (GBPs) that can directly disrupt the vacuolar compartments that contain intracellular pathogens or intersect with autophagy pathways that restrict microbial growth[158,159]. Several GBPs and IRGs are upregulated following *Cryptosporidium* infection[28,68,160,161], but their role in parasite restriction remains largely untested. Although a cluster of GBPs encoded on mouse chromosome 3 is important for resistance to *T. gondii*[162], this particular cluster is not required for early control of *Cryptosporidium*[28]. Whether additional GBPs encoded in a cluster on chromosome 5 have a role remains to be tested. Mice deficient for both IRGM1 and IRGM3 are highly susceptible to *T. gondii* infection[159,163], and although these mice shed more oocysts than wild-type mice when infected with *C. parvum*, they are not as susceptible as IFNγ-deficient mice[28]. These differences in the pathways that mediate resistance to *T. gondii* and *Cryptosporidium* highlight that cell-intrinsic control is not one size fits all. Rather, unique mechanisms may be required for pathogens that occupy specific cell types or distinct subcellular localizations.

IFNγ-dependent, IEC-intrinsic control of *Cryptosporidium* is a complex topic that has not been adequately explored, in large part, because of the reliance on a limited number of transformed human epithelial cell lines. Nevertheless, enterocyte cell lines pre-incubated with IFNβ, IFNγ or IFNλ3 had modest reductions in parasite numbers[68,70,155]. These results appear inconsistent with the potent activity of IFNγ in vivo and emphasize the need for improved in vitro systems to examine the processes that restrict the growth of *Cryptosporidium*. Recent advances using enteric organoid (enteroid) models (Box 1) derived from different hosts, which include mice and humans, have shown that they can be infected with *Cryptosporidium*[164–166]. Whether the host specificity of different *Cryptosporidium* species is a function of their ability to invade IECs and/or the ability of the parasite to evade species-specific mechanisms of resistance (Box 2) remains an open question. As such, the opportunity to access primary cells from a diverse range of hosts provides an important opportunity to understand the mechanistic basis of host susceptibility and resistance to different parasite species.

## Future directions

With tractable models of *Cryptosporidium* infection comes the opportunity to address clinically relevant questions about the relationship between this infection and its effect on malnutrition and development. Clinical data have suggested that recurrent *Cryptosporidium* infections in young children may have a unique effect on nutrition and growth status. In one survey, when compared with all-cause diarrhoeal episodes, only cryptosporidiosis was associated with a decrease in growth progress[10]. Infections in neonatal mice have shown similar effects on mouse growth[167]. Given the important role of the microbiota in immune development during early life, even transient alterations to the microbiome could have long-term effects on immune status[168]. This is exemplified by the report that an attenuated strain of *S.* Typhimurium induces loss of enteric neurons due to neuron-intrinsic NLRP6 inflammasome activation, with persistent changes in intestinal motility[169]. As such, *Cryptosporidium* provides an opportunity to define how infections early in life shape the mucosal landscape during development.

The ability to understand mechanisms of immune activation and control at mucosal sites has been limited by models in which progression to systemic infection can act as a confounding factor and a lack of tractable models for IEC-restricted pathogens. Likewise, although mucosal vaccines have been developed for enteric pathogens such as rotavirus and *S.* Typhimurium, they are almost exclusively live-attenuated-based or reassortant virus-based vaccines, rather than subunit or mRNA approaches[170]. For *Cryptosporidium*, novel vaccination approaches are needed for both animal health and to prevent disease in vulnerable human populations, such as malnourished children, for whom current treatment options have poor efficacy. At present, oral inoculation of calves with irradiated oocysts effectively induces protection[11], whereas the use of *Salmonella* vectors to deliver *Cryptosporidium* antigens in mice has limited efficacy[171]. The capacity to investigate T cell responses to stage-specific antigens, factors that promote T cell-mediated immunity in the small intestine, and the antimicrobial effector pathways that are associated with protective immunity should identify vaccine targets and inform vaccination strategies to develop protective mucosal responses for *Cryptosporidium* and other intracellular enteric pathogens. The ability to compare induction of innate and T cell-mediated immunity across pathogens may additionally provide insights into the contributions of different dendritic cell subsets and how differences in pathogen biology impact these processes. As such, *Cryptosporidium* provides an ideal opportunity to investigate principles of enteric immunity and to apply these lessons to the design of prophylactic strategies to prevent infection.

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## Author contributions

R.D.P. and B.A.W. prepared the initial draft. R.D.P. and C.A.H. revised and edited the manuscript. All authors contributed to the final editing of the manuscript. B.A.W. prepared figures with input from all authors.

## Competing interests

The authors declare no competing interests.

## Additional information

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