Cryptosporidiosis in patients with HIV/AIDS

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Introduction

Cryptosporidium spp. are intestinal protozoan parasites of the phylum Apicomplexa, which cause diarrheal disease in humans worldwide (reviewed in [1–6]). In immunocompetent individuals, infection with this parasite may be asymptomatic or cause a self-limiting diarrheal illness. However, in immunocompromised patients such as those with HIV/AIDS *Cryptosporidium* spp. may cause severe, chronic and possibly fatal diarrhea and wasting. Although *Cryptosporidium* was discovered in 1907, it was not till 1976 that the first human cases of cryptosporidiosis were reported and not till the onset of the AIDS epidemic in the early 1980s that this parasite became widely recognized as a human pathogen (reviewed in [4,7,8]). Indeed, cryptosporidiosis was one of the original AIDS-defining illnesses and as such was associated with an increased risk of death compared to other AIDS-defining illnesses [9]. The use of highly active antiretroviral therapy (HAART) in the past 2 decades has reduced the prevalence of this disease in AIDS patients in industrialized countries [10–12]. However, the emergence of drug-resistant HIV variants and failure or discontinuation of HAART has been associated with re-emergence of *Cryptosporidium* spp. infection in these patients [13,14]. Even patients with advanced AIDS who are on HAART have recently been reported to have *Cryptosporidium* spp. infection [15]. Although antiretroviral treatment (ART) has recently become available in some developing countries it is not widely available or affordable in most areas where the burden of HIV/AIDS is the greatest. In the absence of ART, HIV-infected patients are subject to a cumulative lifetime incidence of diarrhea estimated at up to 100% in developing countries [16] with chronic diarrhea affecting up to 76% of patients with AIDS [17].

In 2004, cryptosporidiosis was added to the WHO's 'Neglected Diseases Initiative' which includes diseases that occur mainly in developing countries and are linked to poverty and lack of access to services [18]. In the absence of a universal treatment program and with the lack of access to care, cryptosporidiosis continues to be a major opportunistic infection and an important cause of morbidity and mortality in persons living with HIV/AIDS in developing countries. Together with the lack of effective specific treatment for cryptosporidiosis in immunocompromised patients, this underscores the critical importance of continuing efforts to develop strategies to prevent and treat this disease in vulnerable populations. Here we review the current state of knowledge about cryptosporidiosis in patients with HIV/AIDS, with a focus on studies from developing countries. In addition, since knowledge of protective immune responses is essential for developing immune-based interventions such as vaccines and immunotherapy, we review what is known about immune responses in cryptosporidiosis, focusing on recent studies.

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Cryptosporidiosis

Prevalence

Cryptosporidiosis is prevalent worldwide and *Cryptosporidium* spp. have been identified in every continent except Antarctica (reviewed in [3,19]). The prevalence of cryptosporidiosis varies widely among different geographic regions and populations at risk and depends on the diagnostic method used to identify the parasite. Seroprevalence studies suggest much higher rates of infection than reports based on detection of the parasite in the stool [3] and range from 30 to 89% (depending on age, geographic location and drinking water source) even in industrialized countries such as the USA [20–22]. Although prevalence rates of cryptosporidiosis have fallen in some industrialized countries such as the UK, following implementation of new drinking water regulations [23], in the USA, *Cryptosporidium* spp. infections have recently been reported to be increasing, most likely due to increased detection of recreational water outbreaks [24]. In patients with HIV/AIDS, reported prevalence rates of cryptosporidiosis also vary widely, ranging from 0 to 100% (reviewed in [19]) with the higher rates reported before ART was in widespread use or from developing countries where ART is not available. However, it is not clear what proportion of the difference in estimates is contributed by differences in study design, geographical location, population group, sensitivity of laboratory methods, or stage of disease.

Most studies on cryptosporidiosis in developing countries have been carried out on HIV-infected adults and employed microscopy on modified acid fast staining of direct or concentrated stool sample preparations for diagnosis [25]. A limited number of studies have used ELISA and PCR for detection [25,26], and found a higher sensitivity for PCR and lower for antigen detection ELISA, compared to microscopy. The data on prevalence in studies using these methods are highly varied. The wide variation in estimates could also reflect issues of study design, with prevalence rates of more than 70% coming from studies with small sample sizes, geographical differences that could affect exposure, as well as differences in the populations being studied, especially with respect to socioeconomic status and access to potable water. The high prevalence of *Cryptosporidium* spp. in AIDS patients in developing countries is probably related to an increased risk of acquiring infection from infected contacts and prolonged excretion, which in turn increases the risk of subsequent transmission. In patients with HIV/AIDS the risk of acquiring cryptosporidiosis is associated with the degree of immunosuppression as measured by CD4 cell counts [9,27–29] (Table 1). Other risk factors for cryptosporidiosis in patients with HIV/AIDS include sex, age, ethnicity and sexual practices [9,29–33].

Table 1. Risk factors for cryptosporidiosis.

Risk factor for cryptosporidiosis	References
General risk factors	
Ingesting contaminated recreational or drinking water	[126,127]
Drinking well water	[128]
Swimming in fresh water or public pools	[129,130]
Eating contaminated food	[131]
Contact with a child in diapers, or in a child care program	[127]
Absence of toilet facilities	[132]
Contact with infected persons	[126]
Contact with farm or domestic animals	[133,134]
Contact with nondomestic animals	[135]
International travel	[136]
Serum MBL deficiency, MBL-2 polymorphisms	[81]
Risk factors in HIV/AIDS	
CD4 cell count <200 cells/μl	[27,33]
Younger age	[29,33]
Male sex	[29]
White race	[30]
Anal sex	[31,32]
Having more than one sexual partner	[31]
Attending a sex venue	[32]

MBL, mannose binding lectin.

Transmission

Cryptosporidiosis is transmitted via the oral–fecal route either by ingestion of contaminated water or food or by direct person-to-person (anthroponotic) or animal-to-person (zoonotic) contact [3,34–37]. The infectious dose depends on the infecting strain, with as few as 10 oocysts sufficient to cause infection [38]. Cryptosporidiosis is largely a water-borne infection and *Cryptosporidium* spp. have been the causative agents of numerous outbreaks of waterborne illness worldwide [34]. Oocysts, the resilient, infectious stage of the parasite are resistant to chlorination and can survive in water, for prolonged periods of time. In industrialized countries, *Cryptosporidium* spp. are one of the commonest causes of water-borne outbreaks of diarrheal disease [19,34] and were the causative agents of the largest ever documented outbreak of waterborne disease in the world, which affected an estimated 403 000 people in Milwaukee, Wisconsin in 1993 [39]. Most of the deaths related to this outbreak occurred in AIDS patients [40]. Recently, most water-borne outbreaks of cryptosporidiosis in the USA have occurred due to contamination of recreational water sources such as swimming pools and water parks [24]. Because of the potential for intentional contamination of water supplies, *Cryptosporidium* is listed as a Category B Priority Pathogen for Biodefense by the US Centers for Disease Control and the National Institutes of Health [41].

Clinical features, diagnosis and treatment

The clinical features of cryptosporidiosis vary widely depending on the immune status of the host (reviewed in [2,3]) (Table 2). In immunocompetent individuals many *Cryptosporidium* spp. infections are asymptomatic, as suggested by the high rates of seropositivity (as high as 89% in some parts of the USA [21]) in the general population. The incubation period for symptomatic cryptosporidiosis is 1–2 weeks and watery diarrhea is the commonest symptom, but abdominal pain, nausea,

Table 2. Clinical features of cryptosporidiosis.

	Immunocompetent	Immunocompromised (AIDS)
Type of illness	Asymptomatic or Mild to moderate	Depends on immune status Can be severe, protracted
Duration	1–2 weeks	Weeks to months
Outcome	Self-limited	Chronic, can be fatal
Symptoms	Watery diarrhea Abdominal cramps Nausea Vomiting Fever	Severe watery diarrhea or Chronic bulky, frequent stools Dehydration Weight loss Wasting
Biliary and pancreatic disease	None	May occur with advanced AIDS Cholecystis, sclerosing cholangitis, pancreatitis
Respiratory disease	Children in developing countries	May occur with advanced AIDS

vomiting and fever may also occur. The illness is self-limited and generally resolves after 1–2 weeks. However, relapse of symptoms following an asymptomatic interval has been reported [3]. Cryptosporidial infection in children in developing countries, particularly in those who are malnourished, often results in persistent diarrhea and may lead to growth faltering, and physical and cognitive impairment (reviewed in [4]).

In patients with HIV/AIDS, clinical manifestations vary with the degree of immune compromise (reviewed in [3,19,42]). Those with CD4 cell counts above 180–200/μl may be asymptomatic or develop self-limiting diarrheal illness. However, patients with advanced AIDS (CD4 cell counts <50/μl), particularly in developing countries where ART is not available, can have severe diarrhea that can persist for several months, resulting in severe dehydration, weight loss and malnutrition, extended hospitalizations, and mortality. In addition, patients with advanced AIDS are at greater risk of developing extraintestinal infection, particularly of the biliary, pancreatic and respiratory tracts (reviewed in [3,6,19]). *Cryptosporidium* spp. are the most commonly isolated pathogens in the biliary tract in patients with AIDS-associated cholangiopathy [43]. Recently, respiratory cryptosporidiosis has been reported in HIV-uninfected children in Uganda, raising the possibility that this complication may occur more commonly in HIV-infected adults as well in this region [44]. AIDS patients with cryptosporidiosis also have a significantly shorter duration of survival from the time of diagnosis [45].

Microscopic detection of oocysts by modified acid fast staining of stool samples (Fig. 1) remains the gold standard for clinical diagnosis of cryptosporidiosis (reviewed in [3,18]). However, this method is less sensitive, laborious, time-consuming and requires a skilled operator to distinguish oocysts from yeast and other debris. Antigen detection assays such as immunofluorescence assays and ELISA, on stool samples are used frequently and are more sensitive and specific than microscopy. However, false-positives have been reported for ELISAs [46]. Although molecular methods such as PCR have been shown to be much more sensitive than other methods for detection of *Cryptosporidium* spp. in stool samples [25,47,48], these assays are not routinely used for clinical diagnosis.

In spite of almost three decades of research on evaluation of over 100 therapeutic agents (reviewed in [49]), nitazoxanide is the only drug that has shown some degree of anticryptosporidial efficacy and has been approved by the Food and Drug Administration for treatment of cryptosporidiosis in immunocompetent persons in the USA [50,51]. Although an intent-to-treat analysis of a compassionate use trial of nitazoxanide in AIDS patients in the US suggested clinical improvement in 59% of those enrolled, a subsequent meta-analysis of seven clinical trials of nitazoxanide indicated that this drug is not effective in immunocompromised individuals [42,52]. A recent double-blind, randomized, placebo-controlled trial of high-dose, prolonged nitazoxanide treatment in Zambian children with AIDS and cryptosporidiosis confirmed the lack of efficacy of this drug in the immunocompromised [53].

Initial treatment of cryptosporidiosis includes fluid and electrolyte replacement and use of antimotility agents (reviewed in [3,5]). Nitazoxanide is recommended in immunocompetent individuals [54] but as discussed above may not be effective in the immunocompromised. In patients with HIV/AIDS, the mainstay of treatment is immune reconstitution with combination ART (cART) which results in clearance or abrogation of infection. Since HIV protease inhibitors have some anticryptosporidial activity *in vitro* and in animal models [55,56], it has been suggested that cART include protease inhibitors [3].

Cryptosporidium spp.

Life cycle

Cryptospordium spp. exist in many intracellular and extracellular developmental stages (Figs 1 and 2). Infection is initiated by ingestion of oocysts either in

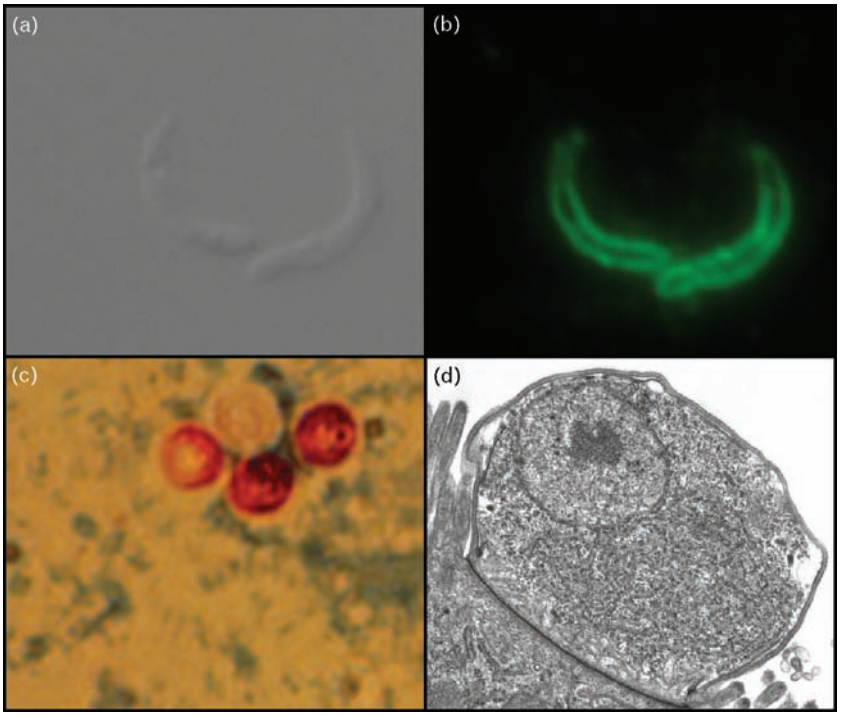

Fig. 1. *Cryptospordium* developmental stages: purified *C. parvum* sporozoites stained by immunofluorescence using a monoclonal antibody CrA1 [152] to gp15, an immunodominant surface antigen. (a) Differential interference contrast (DIC). (b) Immunofluorescence. (c) Modified acid fast staining of *Cryptosporidium* oocysts in stool visualized by light microscopy (reproduced with permission from *Clinical Infectious Diseases*). (d) Intracellular stage (trophozoite) of *C. parvum* visualized by electron microscopy (reproduced with permission from *Microbes and Infection*).

contaminated water or food or by direct person-to-person or animal-to-person contact [1,6]. Oocysts undergo excystation in the small intestine to release sporozoites that attach to and invade the brush border membrane of intestinal epithelial cells. Replication occurs within a parasitophorus vacuole, in a unique intracellular but extracytoplasmic niche, via asexual and sexual cycles. During the asexual cycle, merozoites are released, invade adjacent cells and perpetuate this cycle or differentiate into sexual stages which fuse to form zygotes. These mature into either thick-walled oocysts that are released into the external environment, or thin-walled oocysts that rupture in the intestinal lumen releasing sporozoites that initiate a new round of replication. These thin-walled autoinfective oocysts are believed to contribute to perpetuation of the infection in patients with HIV/AIDS [57].

Species and subtypes

There are two major *Cryptosporidium* spp. which cause human infections (Table 3); *C. hominis* primarily infects humans and *C. parvum* infects humans as well as other animals (reviewed in [58]). Other species that may infect humans include *C. wrairi*, *C. meleagridis*, *C. felis*, *C. saurophilum*, *C. baileyi*, *C. muris*, *C. andersoni*, *C. serpentis* and *C. nasorum* as well as cervine and rabbit genotypes. In developing countries *C. hominis* is the most commonly identified species (reviewed in [58]).

Based on significant variation at a few polymorphic loci, *Cryptosporidium* spp. have been further classified into subtypes [58]. The most commonly used tool for subtyping is based on polymorphisms in the gene encoding gp40/15 (also called gp60), a major surface glycoprotein (reviewed in [58]). Although many different *Cryptosporidium* spp. and subtypes have been identified in patients with HIV/AIDS, particularly those from developing countries (Table 4), there is no particular predilection for infection with any particular species in these patients [58]. However, in recent studies from India and Peru, HIV-infected patients were found to be infected with a greater diversity of species and subtypes compared to immunocompetent individuals in the general population in the community [59–62]. In addition, the clinical manifestations of cryptosporidiosis in HIV-infected patients in developing countries appear to vary depending on the infecting species or subtype. Patients with HIV/AIDS in India who were infected with zoonotic species of *Cryptosporidium*, were more likely to have diarrhea and fever than those infected with *C. hominis* [63]. A study of HIV-infected patients from Tanzania showed that *C. hominis* infection was associated with a longer duration of symptoms, a higher rate of asymptomatic infection, and a lower CD4 cell count compared to *C. parvum* infection [64]. In HIV-infected patients in Peru, individuals infected with *C. parvum, C.*

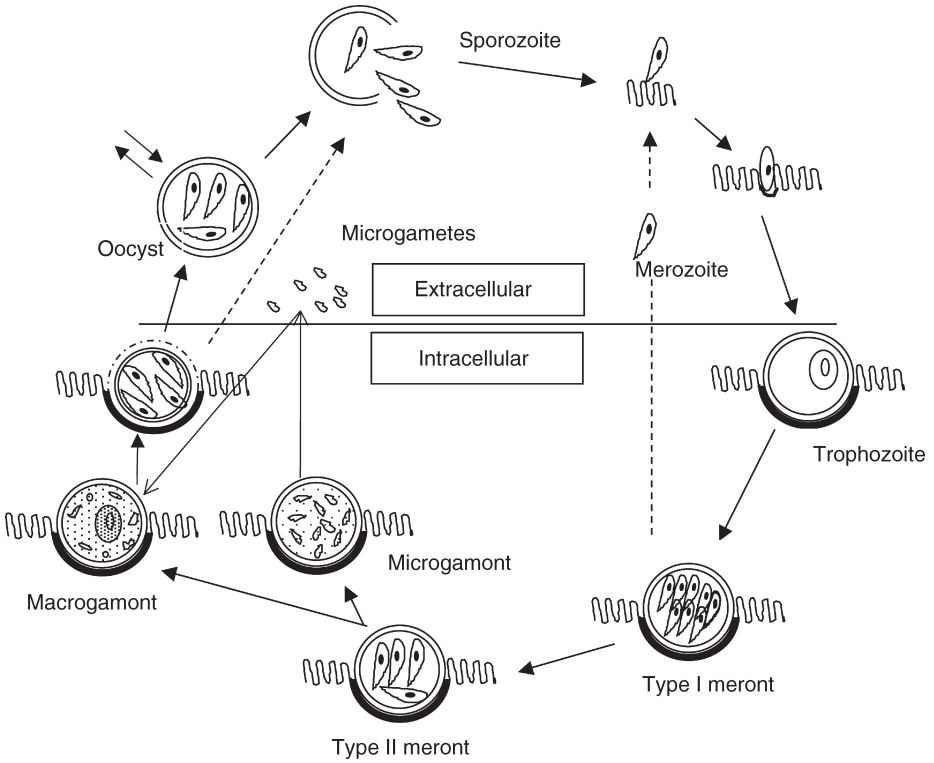

Fig. 2. Life cycle of *Cryptosporidium*. Infection is initiated by ingestion of occysts which excyst to release sporozoites. Sporozoites attach to and invade intestinal epithelial cells in which they develop into trophozoites and then meronts. Merozoites released from type I meronts into the intestinal lumen invade adjacent epithelial cells. Type I meronts further develop into type II meronts which differentiate into macrogamonts and microgamonts. Microgametes released into the lumen fuse with macrogamonts to form zygotes which differentiate into oocysts. Modified with permission from *Advances in Parasitology*.

canis, C. felis, and *C. hominis* subtype family Id were more likely to have chronic than acute diarrhea compared to those infected with other subtypes [62].

Immune responses

Although the immune status of the host plays a critical role in determining the outcome and severity of cryptosporidiosis, immune responses to *Cryptosporidium* spp. are still not completely understood. Much of what is known comes from studies in animal models (reviewed in [65,66]) and in experimentally infected human volunteers with a few studies in naturally infected humans (reviewed in [67]). Overall, these studies demonstrate that both innate and adaptive immune responses are essential for protection from and resolution of infection and that $CD4^+$ T cells and the cytokine IFNγ are of primary importance in anticryptosporidial immune responses (Table 5).

Innate immune responses

IFNγ

The inherent resistance of mice to *C. parvum* infection can be attributed to an early IFNγ response, and even temporary neutralization of IFNγ with a neutralizing antibody renders mice susceptible to infection [65,68] severe combined immunodeficiency disease (SCID) mice develop a chronic infection 18–30 days post infection, but if IFNγ production is disrupted in this background, the mice develop an acute, overwhelming infection [69]. Mice produce IFNγ within 24 h of *C. parvum* infection,

Table 3. *Cryptosporidium* species and subtypes infecting humans.

	gp40/15 (gp60) subtypes
Major species	
C. parvum	**IIa**[a], IIb, **IIc**, **IId**[a], IIe, IIf, IIg, IIh, IIi, IIk, IIl, IIm, IIn
C. hominis	Ia, **Ib**, Ic, Id, Ie, If, Ig
Minor species	
C. felis, C. canis, C. meleagridis, C. suis, C. baileyi, cervine genotype	

The most commonly observed subtypes in humans worldwide are indicated in bold.
[a]Zoonotic potential.

Table 4. *Cryptosporidium* species in patients with HIV/AIDS in developing countries.

City/country	Year[a]	Number[b]	Infecting species	Reference
Equatorial Guinea	2009	35/185 (18.9%)	*C. parvum* (52.9%), *C. hominis* (44.1%), *C. meleagridis* (2.9%)	[137]
Iran	2008	21/35 (60%)	*C. hominis* (79%) *C. parvum* (21%)	[138]
Lop Buri, Thailand	2008	13/46 (28%) 2[c]	*C. parvum* (100%)	[139]
Sao Paulo, Brazil	2008	5	*C. meleagridis* (40%) *C. parvum* (40%)	[140]
Kingston, Jamaica	2008	35	C. hominis (71.4%), C. parvum (20%), C. canis (2.8%), and C. felis (2.8%)	[141]
Venda, South Africa	2006	44/244 (18%)	*C. hominis* (82%), *C. parvum* (18%)	[142]
Lima, Peru	2007	230/2490 (9.2%)	*C. hominis (73%), C. parvum (11.4%), C. meleagridis* (8.8%), C. canis (3.1%), and C. felis (3.1%), *C. suis* (.5%)	[62]
Spain	2007	16	*C. hominis (62%), C. parvum (38%),*	[143]
Port-au-Prince, Haïti	2006	158/1529 (9.9%) 69[c]	*C. hominis* (59%), *C. parvum* (38%) *C. felis* (3%)	[144]
Vellore, India	2006	48/534 (8.9%)	*C. hominis* (64.5%), *C. parvum* (18.7%), *C. felis* (10.4%), *C. parvum* other (2%), *C. meleagridis* (2%), *C. muris* (2%)	[61]
Kampala, Uganda	2005	67/91 (73.6%)	*C. hominis* (74%), *C. parvum* (17%)	[145]
Kilimanjaro, Tanzania	2005	22/127 (17.3%)	*C. hominis* (71.4%), *C. parvum* (28.6%)	[64]
Lima, Peru	2003	354/2672 (13.3%)	*C. hominis* (67.5%), *C. meleagridis* (12.6%), *C. parvum* (11.3%), *C. canis* (4.0%), *C. felis* (3.3%), pig genotype (0.5%)	[146]
Kenya, Malawi, Vietnam, Brazil,	2003	63	*C. hominis* (75%), *C. parvum* 21.7%) *C. meleagridis* (1.6%), *C. muris* (1.6%)	[147]
Thailand	2002	34	*C. hominis* (50%), *C. parvum* (14.7%), *C. meleagridis* (20.6%), *C. felis* (8.8%) *C. canis* (5.9%)	[148]
Durban, South Africa	2002	20	*C. hominis* (76.2%) *C. parvum* (23.8%)	[149]
Bangkok, Thailand	2002	29	*C. hominis* (82.7%), *C. meleagridis* (10.3%), *C. muris* (3%) and *C. felis* (3%)	[150]
Kenya	2000	3	*C. hominis* (100%)	[151]

[a]Year of publication.
[b]Number of *Cryptosporidium* spp. infections detected/number of stool samples tested (%).
[c]Number of samples on which species determination was performed.

Table 5. Immune responses to *Cryptosporidium*.

Innate responses	Adaptive responses
IFN-γ from naive $CD8^+$ iIEL	CD4+αβTCR+
IL-15	CD8+ IEL
MyD88, TLR2, TLR4	IFN-γ, IL-4, IL-5, IL-10, IL-12, IL-18, IL-23
IL-8	Serum IgG, IgA and IgM
β-defensin 2	Fecal IgA
Mannose binding lectin	

from naive $CD8^+$ intestinal intraepithelial cells (iIELs), but the signals initiating this response are unknown [68]. SCID-beige (bg) mice that lack all immune cells except neutrophils and macrophages are resistant to acute infection [70] and it was shown that this protection is conferred by macrophages that have been activated by IFNγ from neutrophils. *In vitro*, IFNγ has been shown to render intestinal epithelial cells resistant to infection [71]. In humans, IFNγ is detected in jejunal biopsies from individuals previously exposed to *Cryptosporidium* spp. but not naive individuals, suggesting a possible mechanism for the differences in resistance between humans and mice [72]. Interestingly, *C. parvum* infection down-regulates IFNγ-induced protein expression in epithelial cells, possibly by suppression of signal transducer and activator of transcription (STAT) 1α signaling [73], In the absence of IFNγ, expression of IL-15 in the jejunal mucosa of human volunteers was associated with a reduced parasite burden [74] suggesting that in humans, this cytokine may be involved in IFNγ-independent control of infection via activation of innate immunity.

Toll-like receptor-mediated pathways

Like many protozoal parasites *Cryptosporidium* spp. activate the Toll-like receptor (TLR) pathway. *In vitro*, *Cryptosporidium* spp. infection of human cholangiocytes activates TLR signaling pathways through both TLR2 and TLR4, resulting in release of IL-8 and human β defensin-2 (HBD-2) [75]. Inhibition of TLR2 and 4 signaling with a MyD88-dominant negative construct resulted in increased numbers of parasites in the infected cells, suggesting that the TLR signaling pathway contributes to suppression of parasitemia. *Cryptosporidium* spp. induction of TLR expression is associated with a reduction in the micro-RNA let-7i [76]. These in-vitro observations are corroborated by studies showing that MyD88 knockout mice are significantly more susceptible to infection than their wild-type littermate controls [77].

Mannose binding lectin

Mannose binding lectin (MBL), an activator of the alternative complement pathway, also plays a role in the innate immune response to *Cryptosporidium* spp. [78]. AIDS patients from Zambia were at significantly increased risk of cryptosporidiosis if they carried homozygous mutations in the *MBL-2* gene [79], and low serum MBL levels in Haitian children were associated with *Cryptosporidium* spp. infections [80]. Likewise, an increased risk for recurrent infections was associated with low serum MBL levels and *MBL-2* polymorphisms in Bangladeshi children [81]. However, MBL $A/C^{-/-}$ mice were not susceptible to cryptosporidiosis [82], suggesting that alterations in MBL levels may only contribute to susceptibility in the presence of other immune deficiencies.

Adaptive immune responses

Cellular immune responses

The importance of $CD4^+$ T-cell-mediated immune responses in resolution of *Cryptosporidium* spp. infections has been clearly established. In humans, this is best illustrated by the increased susceptibility of AIDS patients to infection with this parasite and the observation that AIDS-associated cryptosporidiosis resolves with the restoration of $CD4^+$ T cells following ART [12,13,45,83]. Studies of cryptosporidiosis in immunocompromised mouse models offer further support of this hypothesis (reviewed in [65,66]). T cells from mesenteric lymph nodes (MLNs) and spleens of infected mice, and peripheral blood mononuclear cells (PBMCs) from humans exposed to *Cryptosporidium* spp. proliferate and secrete cytokines upon exposure to crude preparations of *C. parvum* oocysts and sporozoites or recombinant proteins [84–90]. The responding cell population (when identified) is predominantly $CD4^+$, $\alpha\beta$ TCR^+ [84,86,89]. Although not essential, $CD8^+$ T cells, particularly in the IEL population, may also contribute to control of infection [68,91–93] CD40–CD40L interaction is also essential for clearance of *C. parvum* in mice and in humans [94,95]. Individuals with X-linked hyper IgM syndrome, caused by mutations in the CD40L gene, are highly susceptible to disseminated cryptosporidiosis [96,97].

Cytokines

Whereas IFNγ is of primary importance in resistance to and resolution of *C. parvum* infections in mice [90,98] complete immunity to cryptosporidiosis involves a complex balance of Th1 and Th2 cytokines [99,100]. Studies using various knockout mice have demonstrated that IL-4, IL-12, IL-18 and IL-23 all contribute to control of *C. parvum* infections [100–104].

In human volunteers challenged with *C. parvum*, expression of IFNγ and IL-4 in jejunal biopsies was associated with prior exposure to the parasite [74], and IFNγ expression was associated with an absence of oocyst shedding [72]. T-cell clones responsive to *Cryptosporidium* spp. antigens were isolated from PBMCs of healthy individuals with prior cryptosporidiosis [86]. All clones were $CD4^+$ $\alpha\beta TCR^+$ $CD45RO^+$ (memory phenotype) and exhibited hyper production of IFNγ upon antigen stimulation. Some of the T-cell clones exhibited a Th0 phenotype, secreting IL-4, IL-5 or IL-10 in addition to IFNγ. Preincubation of human intestinal epithelial cells

with IFNγ *in vitro* significantly reduces *C. parvum* infection of the cells, and this effect is potentiated by IL-4 [71,105].

Humoral immune responses

The protection conferred by serum and mucosal antibody responses that arise following *Cryptosporidium* spp. infection is unclear. In mice, B cells are not required for resistance to, or resolution of infection [106]. In humans, the presence of pre-existing antibodies to specific antigens correlates with resolution of infection and protection from subsequent challenge [107–109]. However, it is not clear whether the antibody responses are themselves protective or whether they are simply markers of protective cellular responses [66]. Passive transfer of anti-*C. parvum* monoclonal antibodies [66], immune colostrum [110–112] or egg yolk antibody [113,114] can reduce infection, protect against the development of diarrhea and reduce oocyst shedding, but does not eliminate the infection.

In the pre-ART era, many attempts were made to treat AIDS-associated cryptosporidiosis with bovine colostrum with limited success [110,115–117] and a trial of hyperimmune bovine colostrum in human volunteers challenged with *C. parvum* had no significant effect on the course of infection though there was a trend towards less diarrhea in patients treated with colostrum [118]. However, it is perhaps important to note that the most efficacious preparations in the animal studies were generated with individual recombinant *C. parvum* proteins [112,119,120]; the efficacy of such preparations in humans has not been investigated.

The immune response to cryptosporidiosis in patients with HIV/AIDS

CD4 T cells are clearly of prime importance in clearing cryptosporidiosis, therefore the susceptibility of AIDS patients to cryptosporidiosis is not surprising. There are certain aspects of the host–parasite interaction that perhaps further contribute to the particular susceptibility of AIDS patients to cryptosporidiosis (given below).

Immune responses compromised by HIV/AIDS that contribute to susceptibility to *Cryptosporidium*:

(1) Depletion of lamina propria $CD4^+$ T cells
(2) Poor proliferation and altered cytokine secretion of PBMCs
(3) Increased CXCL10 expression in *Cryptosporidium*-infected intestinal epithelial cells
(4) Inhibition of TLR4 expression by HIV tat protein

Lamina propria $CD4^+$ T cells are the first to be depleted in HIV [121], and recovery of an AIDS patient from cryptosporidiosis was associated with rapid repopulation of the mucosa with $CD4^+$ lymphocytes [122]. PBMCs from HIV-positive individuals with cryptosporidiosis, stimulated with *C. parvum* antigens, exhibit poor proliferative responses and altered cytokine production [85,123]. In AIDS patients with active cryptosporidiosis, infected epithelial cells express high levels of the chemokine, CXCL10, and expression levels correlate with the parasite burden [124]. Upon immune reconstitution, the levels of CXCL10 declined, and intestinal CD4 T cells increased. Since CXCL10 increases the rate of HIV replication *in vitro*, it was suggested that elevated CXCL10 in cryptosporidiosis may contribute to HIV destruction of $CD4^+$ T cells. Innate immune responses may also be affected. Inhibition of TLR4 expression in cholangiocytes with HIV tat protein resulted in increased parasite numbers, suggesting that this may contribute to the particular susceptibility of AIDS patients to biliary cryptosporidiosis [125].

Conclusion

Although, with the widespread use of effective ART, cryptosporidiosis is no longer the devastating illness it once was in AIDS patients in developed countries, it continues to pose a major threat to AIDS patients in resource-poor developing countries where ART is not widely available or affordable. Since there is currently no effective specific therapy or vaccine available for cryptosporidiosis in the immunocompromised, it is imperative to continue investigation into the biology of *Cryptosporidium* and host immune responses to it in order to develop novel and effective prophylactic and therapeutic strategies to prevent and treat this disease in those who are at the greatest risk of acquiring it and suffering its consequences.

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