Published in final edited form as:

Future Microbiol. 2010 March ; 5: 507–519. doi:10.2217/fmb.09.128.

Human immune responses in cryptosporidiosis

Anoli Borad and

Division of Internal Medicine, Section of Infectious Diseases, Yale University, 300 Cedar Street, TAC S169, New Haven, CT 06520, USA, Tel.: +1 203 737 5847, Fax: +1 203 785 6815, anoli.borad@yale.edu

Honorine Ward[†]

Division of Geographic Medicine & Infectious Diseases, Tufts Medical Center, Box 41, 800 Washington Street, Boston, MA 02111, USA, Tel.: +1 617 636 7022, Fax: +1 617 636 5292, hward@tuftsmedicalcenter.org

Abstract

Immune responses play a critical role in protection from, and resolution of, cryptosporidiosis. However, the nature of these responses, particularly in humans, is not completely understood. Both innate and adaptive immune responses are important. Innate immune responses may be mediated by Toll-like receptor pathways, antimicrobial peptides, prostaglandins, mannose-binding lectin, cytokines and chemokines. Cell-mediated responses, particularly those involving CD4⁺ T cells and IFN- γ play a dominant role. Mucosal antibody responses may also be involved. Proteins mediating attachment and invasion may serve as putative protective antigens. Further knowledge of human immune responses in cryptosporidiosis is essential in order to develop targeted prophylactic and therapeutic interventions. This review focuses on recent advances and future prospects in the understanding of human immune responses to *Cryptosporidium* infection.

Keywords

antibody; cell-mediated immunity; chemokine; cryptosporidiosis; *Cryptosporidium*; cytokine; diarrhea; humoral immunity; parasite; T cell

Cryptosporidium is an apicomplexan protozoan parasite that causes diarrheal disease in humans worldwide. The biology, pathogenesis, epidemiology and clinical features of *Cryptosporidium* and cryptosporidiosis have been comprehensively described in several excellent reviews within the past 10 years [1-12] and are briefly summarized here.

The immune status of the host plays a critical role in determining susceptibility to infection with this parasite as well as the outcome and severity of cryptosporidiosis. In immunocompetent hosts infection is often asymptomatic or mild to moderate and self-limited (reviewed in [11]). However, in immunodeficient hosts such as patients with HIV/AIDS, congenital immunodeficiencies and transplant recipients, infection can result in persistent, debilitating and possibly fatal diarrhea and wasting (reviewed in [10,11]). In areas where cryptosporidiosis is endemic, most symptomatic infections occur in early childhood and in the immunodeficient [4]. Although *Cryptosporidium* primarily infects the distal small

intestine, in severely immunodeficient patients this parasite can infect extraintestinal sites such as the lungs, biliary tract and pancreas (reviewed in [11]).

Transmission of the parasite occurs via the fecal–oral route, either by ingestion of contaminated water or food or by person-to-person or animal-to-human transmission (reviewed in [11,12]). A major mode of transmission is via contaminated water supplies, often resulting in widespread outbreaks (reviewed in [12]). While cryptosporidiosis can be endemic in developing countries, several epidemic waterborne outbreaks have also been reported in developed countries. The potential for intentional contamination of water supplies has led to inclusion of *Cryptosporidium* as a Category B priority pathogen for biodefense [13].

There are two major species of *Cryptosporidium* that infect humans. The zoonotic species *Cryptosporidium parvum* infects animals as well as humans whereas the anthroponotic species *Cryptosporidium hominis* primarily infects humans (reviewed in [8]). Other species that have occasionally been reported to infect humans include *Cryptosporidium felis*, *Cryptosporidium meleagridis*, *Cryptosporidium muris*, *Cryptosporidium wrairi*, *Cryptosporidium saurophilum*, *Cryptosporidium baileyi*, *Cryptosporidium andersoni*, *Cryptosporidium serpentis* and *Cryptosporidium nasorum* (reviewed in [8]).

After ingestion of as few as nine oocysts (reviewed in [2]), sporozoites are released from excysted oocysts into the intestinal lumen and invade epithelial cells, particularly in the terminal ileum [3,11]. The parasite then undergoes replication via asexual and sexual cycles within a parasitophorous vacuole that is located in the intestinal epithelial cell membrane in an intracellular yet extracytoplasmic location. Following sexual reproduction, large numbers of both thick- and thin-walled oocysts are released. Thin-walled oocysts excyst within the lumen to infect other epithelial cells resulting in an autoinfection cycle, while thick-walled oocysts are excreted in the feces [11]. Infection of the gut epithelium may result in villus flattening, which causes malabsorption and diarrhea [11]. In addition, there may be a secretory component to the diarrhea which may be due to increased substance P [14] or prostaglandin production [15], and disruption of the intestinal epithelium, which can inhibit NaCl absorption. The parasite may promote apoptosis in adjacent epithelial cells while inhibiting apoptosis in the infected cells, thereby facilitating prolonged survival of the parasite [16]. In immunocompetent persons, *Cryptosporidium* was reported to account for up to 6.1% of diarrheal disease worldwide (reviewed in [2]). However, these studies used stool microscopy for detection of infection, whereas recent studies using PCR for detection suggest that the number of infections may actually be higher [17]. The incubation period to establish infection can range from 1 to 2 weeks (reviewed in [10]). Most patients with symptomatic infection present with acute watery diarrhea that lasts for a few days to 2 weeks, but can be persistent and last for up to 5 weeks. Other accompanying symptoms may include nausea, vomiting, anorexia, abdominal cramping, fever and weight loss.

Cryptosporidiosis is a common cause of parasitic diarrhea in patients with HIV/AIDS and was reported to occur in up to 24% of these patients in the era before highly active antiretroviral therapy (HAART) was available (reviewed in [2]). In patients with HIV/AIDS the severity of the disease varies, depending on the degree of immunosuppression, as reflected by CD4⁺ counts. In patients with relatively high CD4⁺ counts (>180 cells/mm³) the infection may be asymptomatic or result in mild diarrhea. However, patients with CD4⁺ counts of less than 50 cells/mm³ can develop persistent or intractable diarrhea, thus emphasizing the importance of the host immune response in controlling the disease (reviewed in [4]).

Cryptosporidium is a major cause of diarrhea in children in developing countries, particularly in those under 2 years of age. Up to 12% of diarrheal disease in children in these countries may be caused by this parasite [11]. In developing countries, cryptosporidiosis is more common, and its consequences are more severe in malnourished than well-nourished children, possibly because of impaired T-cell responses [18]. Cryptosporidiosis in early childhood can lead to growth faltering and impaired cognitive development, as well as further malnutrition which can put these children at higher risk for recurrent diarrheal diseases. A recent investigation suggests a possible genetic predisposition to cryptosporidiosis in Bangladeshi children with the *B*15* HLA I allele and the *DQB1*0301* HLA II allele [19].

In developed areas of the world, the prevalence of cryptosporidiosis in patients with HIV/AIDS has declined with implementation of strategies to improve water supplies and the institution of HAART. However, the emergence of drug-resistant HIV variants and failure or discontinuation of HAART is associated with the re-emergence of *Cryptosporidium* infection [20,21]. Furthermore, HAART is not widely available or affordable to patients in most developing countries. Currently available therapy for cryptosporidiosis is suboptimal. Although over 100 therapeutic agents have been evaluated for anticryptosporidial efficacy, nitazoxanide is the only drug that has been approved by the US FDA for treatment of cryptosporidiosis in immunocompetent persons in the USA. However, this drug is not effective in immunocompromised individuals [22,23]. In patients with HIV/AIDS, the mainstay of treatment is immune reconstitution with HAART, which results in clearance or abrogation of infection. There is no vaccine available for cryptosporidiosis.

The persistent threat posed by *Cryptosporidium*, the paucity of effective chemotherapeutic agents and lack of a vaccine underscores the importance of continuing efforts to identify specific target antigens and better understand host immune responses to the parasite in order to develop effective immune-based prophylactic and/or therapeutic strategies.

Immune response

Although the outcome and severity of infection is critically dependent on the immune status of the host, the nature of the immune response in cryptosporidiosis, particularly in humans, is poorly understood. Much of what is known about immune responses to *Cryptosporidium* is based on studies done in animals, particularly in mice (reviewed in [24,25]). However, there have been very few studies on immune responses in humans. Most studies in humans have focused on systemic antibody responses with a few addressing cell-mediated responses. Other than fecal antibody responses, there have been no studies on mucosal immune responses in humans. Such studies are challenging to perform in humans since they would involve invasive tissue sampling. Thus, most investigations have been done in tissue culture models utilizing human cell lines, or on peripheral blood mononuclear cells (PBMCs). *In vitro* studies in cancer-derived human cell lines and *ex vivo* studies on peripheral blood mononuclear cells have limitations since they may not accurately reflect the intricate dynamics of the mucosal immune system *in vivo* [26]. In this review we have focused mainly on studies of immune responses in humans and have also included *in vitro* studies in human cell lines.

Innate immune responses

Recent studies in humans as well as *in vitro* in human cell lines suggest that specific innate immune responses may play a role in resistance to cryptosporidiosis. Studies done in humans are summarized in Table 1.

Toll-like receptor-mediated pathways—Toll-like receptors (TLRs) are a family of conserved molecules that play an important role in mediating resistance to a wide array of pathogens by recognizing specific pathogen-associated molecular patterns (PAMPs) [27,28]. Most TLRs signal by recruiting MyD88, an adaptor protein which is involved in stimulation of several pathways of the innate immune system, including the IL-1 receptor, IL-18 receptor and TLR pathways [29,30]. Recognition of PAMPs by TLRs and subsequent recruitment of MyD88 activate a cascade of kinases, resulting in the nuclear translocation of NF- κ B, the expression of proinflammatory cytokine genes and the initiation of host immune responses. Recent studies have shown that *C. parvum* induced recruitment of TLR2 and TLR4 to the site of infection leading to activation of downstream effectors in a human cholangiocyte model *in vitro* [31]. Knockdown of MyD88, TLR2 and TLR4 expression using dominant negative mutant and siRNA approaches resulted in inhibition of downstream signaling pathways and increased *C. parvum* infection in cells in which MyD88 expression was decreased. TLR4 expression in this model appears to be regulated by the miRNA let-7. *C. parvum* infection resulted in MyD88 and NF- κ B-dependent suppression of let-7 and consequent upregulation of TLR4 in these cells [32].

These *in vitro* studies were performed in human cholangiocytes, which are not the primary site of infection. However, there have been no studies reported on the role of TLR-mediated pathways in *C. parvum* infection in intestinal epithelial cells or other immune cells that the parasite or its antigens may come in contact with, such as dendritic cells and macrophages. Although TLR ligands for *Cryptosporidium* have not been identified, the parasite expresses surface-associated glycosylinositol phospholipids [33] and glycosylphosphatidylinositol anchors [34], which have been implicated as putative TLR ligands in other apicomplexan parasites such as *Plasmodium* [35].

Antimicrobial peptides—With improved understanding of mucosal defense it is evident that the intestinal mucosa not only serves as a physical barrier, but plays an active and important role in innate defenses. Antimicrobial peptides (AMPs) are one of the components of the intestinal mucosal barrier. AMPs are small polypeptides (<100 amino acids) that have antimicrobial and immunomodulatory properties and are evolutionarily conserved effectors of the innate immune system [36]. AMPs that are expressed and secreted by human intestinal epithelial cells include α - and β -defensins and cathelicidins. There are six known human β -defensins (HBDs), of which HBD-1 is constitutively expressed and HBD-2, -3 and -4 are expressed during infection and/or inflammation.

In vitro studies in human intestinal epithelial cell models have shown that *C. parvum* infection initially downregulates HBD-1 production, perhaps thus facilitating parasite survival [37]. However, infection also induces expression and secretion of HBD-2, which has antimicrobial activity against the parasite *in vitro* and may play a role in recruitment of T cells and dendritic cells *in vivo*, eventually facilitating clearance of the parasite. *C. parvum* has also been shown to upregulate HBD-2 expression via TLR2- and TLR4-mediated signaling and NF- κ B activation in infected human cholangiocytes [31].

Mannose-binding lectin—Mannose-binding lectin (MBL) is another highly conserved component of the innate immune system [38]. It is a collagenous lectin found in serum that binds to specific carbohydrate residues on a variety of infectious organisms including *Cryptosporidium* [39]. Upon binding, MBL activates the lectin complement pathway in an antibody-dependent manner via mannose-binding lectin-associated serine proteases (MASPs), thereby promoting opsonization and phagocytosis. MBL activation is thought to result in formation of a membrane attack complex that may diminish parasite viability in addition to promoting opsonization and phagocytosis [39]. In a study of AIDS patients with cryptosporidiosis, patients homozygous for structural mutations in the *mbi2* gene and

consequent low serum MBL levels were more susceptible to infection with *Cryptosporidium* [39]. Low MBL levels may also be related to malnutrition or protein losses in the gut [39]. A study of Haitian children with cryptosporidiosis, many of whom were malnourished, reported that children with MBL deficiency (MBL ≤ 70 ng/ml) were more likely to be infected with the parasite [40]. However, this study did not investigate polymorphisms in the *mbl2* gene in these children. A recent study in Bangladeshi pre-school children reported that MBL deficiency and *mbl2* polymorphisms were strongly associated with *Cryptosporidium* infection, particularly in those with repeated infections [41]. Further studies are needed to better understand the implications of malnutrition, MBL deficiency, the role of *mbl2* polymorphisms and risk of subsequent infections.

Chemokines—Chemokines are a family of small 8–10-kDa protein molecules that are produced by epithelial cells. They exert their effects by interacting with G-protein-linked transmembrane chemokine receptors and function as chemoattractants for inflammatory cells by inducing chemotaxis and activating leukocytes. They are divided into two major groups, CC chemokines with adjacent cysteine residues, and CXC chemokines with cysteine groups separated by an amino acid. The CC chemokine ligand (CCL)-5 is a potent chemoattractant that is upregulated in a human intestinal epithelial cell model of *C. parvum* infection [42]. A study of *C. parvum* infection in human intestinal epithelial cells and intestinal xenografts reported increased expression of the CXC chemokine ligand (CXCL)-8 (or IL-8) and growth-regulated oncogene (GRO)- α in infected cells [43].

Peripheral blood mononuclear cells of malnourished Haitian children with cryptosporidiosis expressed higher levels of CXCL-8 upon *ex vivo* stimulation with *C. parvum* [44]. This cytokine was also detectable in stool samples of some Brazilian and Haitian children with cryptosporidiosis [45,46]. In addition, the CXC chemokine CXCL-10 (IFN- γ inducible protein 10), which recruits IFN- γ -producing T cells, was expressed in higher levels in jejunal biopsies of AIDS patients with cryptosporidiosis compared with uninfected control AIDS patients or normals [47]. Additional studies are required to confirm the role of specific chemokines in human cryptosporidiosis.

Cytokines—Cytokines are proteins that play a key role in modulation of both innate and adaptive immune responses and are discussed in this section as well as in that on adaptive immune responses. Studies in mice have established that IFN- γ is a major player not only in cell-mediated immunity, but in early innate immune responses as well [24,25,48]. However, the role of IFN- γ in human infections is not as clear. *In vitro* studies using the human intestinal cell lines, Caco-2 and HT-29, have demonstrated that IFN- γ directly prevents the parasite from invading host cells, most likely by activation of the JAK/STAT signaling pathway following binding to IFN- γ receptors on intestinal epithelial cells, as well as by modification of intracellular Fe²⁺ concentrations [49]. A recent study suggested that *C. parvum* infection may downregulate IFN- γ by suppression of STAT1- α signaling, thus facilitating invasion [50].

A case of persistent cryptosporidiosis has been described in a child with primary IFN- γ deficiency [51], suggesting that this cytokine is also important in human infections. In a study of experimentally infected humans, jejunal biopsies from previously uninfected (seronegative) individuals demonstrated no mucosal IFN- γ production, whereas those from most of the seropositive individuals did [52]. In the absence of IFN- γ production, seronegative individuals expressed IL-15, suggesting that other cytokines may play a role as well. Those volunteers that expressed higher levels of IL-15 in their jejunal mucosa had symptomatic infection but shed fewer oocysts than seronegative volunteers that did not express IL-15 [53]. Subsequently, it was demonstrated that IL-15 activates natural killer cells and $\gamma\delta$ -T cells and may assist in recruiting other cells, which leads to clearance of the

parasite [54]. IL-4 was also expressed in the jejunal mucosa from seropositive volunteers, but there was no association with presence of symptoms or oocyst shedding. Jejunal biopsies from some experimentally infected human volunteers also expressed TGF- β [55]. Again, additional studies are essential to define the role of specific cytokines in innate and consequently adaptive immune responses.

Prostaglandins & substance P—Prostaglandins are potent lipid molecules that exert their effects via a wide array of receptors. In a study of human volunteers experimentally infected with *C. parvum*, it was shown that jejunal biopsies expressed TNF- α and IL-1 β , which can stimulate prostaglandin production, but there was no association with symptoms [56]. *C. parvum* infection of human intestinal epithelial cells *in vitro* resulted in activation of prostaglandin H synthase 2 expression and increased production of prostaglandin (PG)-E2 and -F2- α [15]. Although prostaglandins may contribute to the pathogenesis of secretory diarrhea by altering chloride uptake and fluid secretion, they may also upregulate mucin production from epithelial cells, which can protect the host intestinal mucosa from being infected with *C. parvum* by interfering with attachment of the parasite. In addition, prostaglandins may stimulate HBD production and downregulate expression of inflammatory cytokines. Substance P is a neuropeptide that is located in the GI tract as well [57]. It can cause chloride ion secretion and play a role in secretory diarrhea. Jejunal biopsies from AIDS patients with cryptosporidiosis showed increased expression of substance P mRNA and protein in patients with diarrheal symptoms [14]. However, the exact roles of prostaglandins and substance P need to be further defined.

Adaptive immune responses

Cell-mediated immunity & T-cell responses—The crucial role of cell-mediated immune responses in protection from, and resolution of, cryptosporidiosis has been well established in both murine models and human studies [25,58,59]. Recent studies in humans are summarized in Table 2. The increased susceptibility of AIDS patients to *Cryptosporidium* infection and resolution of cryptosporidiosis following immune reconstitution underscores the importance of CD4⁺ T cells [60,61]. Patients with CD4⁺ counts less than 50 cells/mm³ are more likely to have a fulminant form of the disease, while those with CD4⁺ counts of 180 cells/mm³ or more tend to have less severe self-limited disease (reviewed in [4]). CD4⁺ cell responses are mediated in large part by the cytokine IFN- γ [25]. PBMCs from infected humans proliferate in response to recombinant and crude preparations of *Cryptosporidium* antigens [62-65]. The response is MHC II-dependent and is characterized by increased production of IFN- γ [62,63]. T-cell clones derived from PBMCs of *Cryptosporidium*-exposed patients stimulated with native and recombinant antigens were predominantly CD4⁺ TCR- α/β CD45RO⁺ (memory phenotype), and were characterized by hyper-production of IFN- γ . Some of the T-cell clones exhibited a Th0 phenotype, secreting IL-4, IL-5 or IL-10 in addition to IFN- γ [64]. In a recent study, in which whole blood from seropositive and seronegative human volunteers was stimulated *ex vivo* with recombinant *C. hominis* gp15 antigen, both CD4⁺ and CD8⁺ cells from seropositive donors produced greater amounts of IFN- γ than those from seronegative donors [66]. In a human volunteer study of adults experimentally infected with *Cryptosporidium*, mucosal IFN- γ production correlated significantly with the presence of pre-existing anti-*Cryptosporidium* antibodies, and reduction in oocyst shedding, suggesting that prior exposure to *Cryptosporidium* may be important in developing protective IFN- γ -mediated memory responses in subsequent infections [52]. The role of CD8⁺ T cells in protection from or clearance of infection is not clear. However, in the human study referred to above, both CD4⁺ and CD8⁺ T cells secreted IFN- γ in response to *ex vivo* stimulation with recombinant gp15 [66].

Humoral immunity & antibody responses—While the critical role of T-cell-mediated responses in the control of cryptosporidiosis is undisputed, the specific role of humoral immunity is still unclear. Invasive stages of the parasite, including sporozoites and merozoites, when present in the intestinal lumen are the most likely stages to be targeted by specific antibodies, which may function by blocking attachment and invasion of these stages into host cells.

In humans, the increased susceptibility of patients with primary immunodeficiencies, such as X-linked hyper-IgM syndrome, and selective IgA deficiency to cryptosporidiosis [2,67,68] suggest that humoral immunity may play a role. However, many of these individuals may have defects in both B- and T-cell responses. A possible role for antibodies is also supported by the partial efficacy of some hyperimmune bovine colostrum preparations (derived from cows immunized with *C. parvum*) in cryptosporidial infections in healthy human volunteers as well as in AIDS patients (reviewed in [25]). Sera from AIDS patients recognized fewer cryptosporidial antigens when compared with non-AIDS patients, suggesting that deficient CD4⁺ T-cell responses may result in ineffective antibody responses as well [69].

A number of studies have reported the presence of *Cryptosporidium*-specific serum IgG, IgM and IgA, and fecal IgA antibodies following cryptosporidial infection in humans (reviewed in [25]). Many of these have been seroprevalence studies that have reported a wide range of seropositivity depending on age, geographic location, living and environmental conditions. Recent studies of antibody responses to crude or specific native or recombinant *C. parvum* antigens in humans with cryptosporidiosis are summarized in Table 3. A study of mucosal antibody responses in experimentally infected human volunteers found that anticryptosporidial fecal IgA was present in active infection [70]. Studies in human volunteers and serological surveys suggest that individuals with pre-existing antibodies to *Cryptosporidium* may be partially protected and experience less diarrheal symptoms upon subsequent challenge [71-74]. However, it remains to be determined whether antibody responses are themselves protective or whether they are merely markers of a protective cell-mediated response [25].

Cryptosporidium antigens—In order to develop targeted immune-based interventions such as vaccines, or passive immunotherapy for cryptosporidiosis, it is essential to identify and characterize specific antigens that mediate attachment and invasion of the parasite into host cells and which may therefore serve as putative targets for these interventions. Like other apicomplexan parasites, the apical region of *Cryptosporidium* sporozoites and merozoites contains specialized secretory organelles (rhoptries, micronemes and dense granules) collectively known as the apical complex. These organelles secrete and successively exocytose proteins, which facilitate attachment, gliding motility, invasion and parasitophorous vacuole formation. Surface proteins of invasive stages may also mediate attachment. Surface and apical complex proteins, which are believed to mediate attachment and invasion of *Cryptosporidium*, include circumsporozoite-like protein (CSL), gp900, p23/27, TRAP C1 and other TRAPs, gp40/45, cp47, gp15/cp17, Cp2, Cpa 135, Muc4 and Muc5 [3,9,75]. Many of these are mucins or mucin-like glycoproteins. Sequencing of the *Cryptosporidium* genome has facilitated identification of some of these proteins [76,77]. However, progress in establishing their functional role has been hampered by the inability to propagate *C. parvum* *in vitro* and to genetically manipulate the parasite [9]. Characteristics of these proteins have been recently reviewed or published [3,9,75,78,79]. Here we focus on those proteins which have been shown to induce immune responses in humans.

The best studied of these proteins is gp40/15 (also called GP60 or S60), a mucin-like glycoprotein [80-83]. It is encoded by a highly polymorphic gene *Cpgp40/15*, and is expressed as a precursor glycoprotein that is proteolytically processed [84,85] to yield

mature glycopeptides gp40 (also referred to as gp45) and gp15 (also referred to as Cp17/S16/15–17-kDa antigen), which remain noncovalently associated following cleavage [86]. In spite of the polymorphisms in the *Cpgp40/15* gene, the gp15 part of the molecule is relatively conserved. The C-terminal gp15 peptide is anchored to the membrane via a glycosylphosphatidylinositol linkage, localized to the surface of zoites, and is shed in trails during gliding motility [82,83,87,88]. gp15 is an immunodominant protein consistently recognized by sera from infected persons [81]. The presence of pre-existing serum antibodies to gp15 correlated with protection from diarrheal symptoms in infected adult humans [72,74]. In a longitudinal analysis of serological responses in a Peruvian cohort of children, IgG responses to gp15 were detected following infection and tended to be higher in older children, possibly indicating recurrent exposure [89]. In a case–control study of cryptosporidiosis in Bangladeshi children with diarrhea [90], cases had significantly higher IgM levels to gp15 following an episode of cryptosporidial diarrhea compared with controls [91]. In addition, the increase in IgG levels to gp15 after a 3-week follow-up period was significantly greater in cases as compared with controls. The N-terminal gp40 peptide is secreted [82,88] and localizes to the surface of the invasive stages in association with gp15 [92]. gp40 binds to human intestinal epithelial cells, and antibodies to gp40 inhibit *C. parvum* infection *in vitro* [82,88]. Studies in a birth cohort of children in a semi-urban slum area of south India where *C. hominis* subtype 1a infection predominates [93] indicated that IgG responses to this antigen occur following an episode of cryptosporidial diarrhea, and appear to be, at least in part, subtype specific [94]. Cp23 (also called p27 or 27-kDa antigen) is a surface protein expressed on the invasive stages of the parasite, is shed in trails during gliding motility [95] and is also predicted to display mucin-type *O*-glycosylation. Like gp15, Cp23 is an immunodominant protein and antibodies to it are frequently detected following *Cryptosporidium* infection [25]. In a study of experimentally infected human volunteers, those that had pre-existing serum IgG to the 27-kDa antigen excreted fewer oocysts compared with those that did not [72]. In a Peruvian cohort study, as was the case with the 17 kDa antigen, IgG levels to the 27-kDa antigen levels tended to be higher in older children [89]. A study of HIV-infected patients also showed that patients with higher antibody responses to the 27-kDa antigen tended to be asymptomatic [74]. In the case–control study of Bangladeshi children referred to above, there was a significant increase in IgG, IgA and IgM levels to p27 in children with cryptosporidial diarrhea compared with those with diarrhea due to other etiologies [96]. In addition, children with a shorter duration of diarrhea tended to have higher antibody levels to p27. p27 also induced cell-mediated immune responses in PBMCs of infected humans [57]. Although a few polymorphisms have been identified in the *p27* gene, this protein is relatively conserved among different isolates [97]. Neutralization-sensitive epitopes on this protein also appear to be conserved among isolates [95,98].

gp900 is a heavily glycosylated, high molecular weight glycoprotein that is localized to micronemes, secreted onto the surface of sporozoites and shed in trails during gliding motility [99,100]. It is a multidomain protein containing cysteine-rich, mucin-like and transmembrane domains [100]. Purified native gp900 binds to human intestinal epithelial cells and competitively inhibits *C. parvum* infection *in vitro* [100,101]. gp900 displays mucin-type *O*-linked glycosylation and *N*-acetylgalactosamine-containing glycotopes of this protein (which are also present on gp40), are the target of infection-neutralizing lectins as well as of a neutralizing monoclonal antibody [80]. A recombinant 40 kDa fragment of gp900 named surface antigen 40 (SA40) induced proliferative responses and IFN- γ secretion from PBMCs of *Cryptosporidium*-infected immunocompetent (but not immunocompromised) human volunteers [64].

Future perspective

It has been challenging to investigate immune responses, particularly mucosal cell-mediated responses to *Cryptosporidium*, in humans due to the invasive techniques required. In addition, characterization of putative protective antigens and their functional role has been hampered by the inability to propagate the parasite *in vitro* and to genetically manipulate it. Despite these impediments, there has been considerable advancement in our knowledge of human immune responses to *Cryptosporidium*. However, substantial gaps in understanding the nature of immune responses to this parasite still remain.

In future studies, the role of innate immune responses in human subjects could be further explored. The role of TLR-mediated pathways in human intestinal epithelial cells or dendritic cells in innate immune responses to *Cryptosporidium* could be investigated. The association of genetic alterations, such as polymorphisms in TLR genes and susceptibility to cryptosporidiosis in vulnerable populations could be evaluated. TLR ligands for *Cryptosporidium* that may serve as adjuvants, or other similar modulators of immune responses could be identified. Secretion of defensins and/or cathelicidins in response to infection could be studied by measuring fecal levels of these AMPs, as has been carried out in inflammatory bowel disease [102]. Investigation of the role of chemokines and cytokines in innate and adaptive responses in humans could be facilitated by the use of newer technologies that require only small volumes to quantify several cytokines in the same sample.

In order to identify and evaluate putative protective antigens as targets for vaccines or passive immunotherapy, longitudinal studies of cellular and humoral responses to these specific antigens in vulnerable cohorts of human populations are required. In the few studies that have been done to evaluate human responses to specific antigens, *Escherichia coli*-derived recombinant proteins have been used. However, unlike the native proteins, these are not glycosylated. Glycosylated epitopes of these proteins may be required for effective immune responses. Use of heterologous expression systems, such as the *Toxoplasma* system [103], to generate glycosylated recombinant antigens may be required to fully evaluate immune responses to mucin-like glycoproteins, which include most of the currently known *Cryptosporidium* adhesins. Development of robust systems for continuous propagation of *Cryptosporidium in vitro*, and for stable transfection, knockout or knockdown of *Cryptosporidium* genes as well as genomic and proteomics approaches will facilitate identification of new antigens and confirmation of their functional role. A better understanding of the nature and dynamics of secretory mucosal antibody responses, including measurement of fecal and salivary secretory IgA to specific antigens, using highly sensitive newer technology may give more insight into mucosal humoral immune responses.

Despite the numerous advances made in understanding immune responses to *Cryptosporidium*, a significant amount remains unknown. Given the paucity of effective therapeutics, the lack of a vaccine and the challenges in eradicating transmission of this parasite, it is imperative to develop more targeted prophylactic and therapeutic interventions to ameliorate the morbidity resulting from cryptosporidiosis, especially in children and in immunosuppressed populations.

Executive summary

Innate immune responses

Toll-like receptor-mediated pathways

- *In vitro* studies in human cholangiocytes demonstrate that activation of Toll-like receptor-2 and -4 responses leads to chemokine production via a MyD88 dependent NF- κ B pathway.

Antimicrobial peptides

- *Cryptosporidium parvum* infection downregulates expression of the antimicrobial peptide human β -defensin (HBD)-1 and induces HBD-2 expression in human cell lines *in vitro*.

Mannose-binding lectin

- Low serum mannose-binding lectin (MBL) levels and *mbi2* polymorphisms are associated with increased susceptibility to cryptosporidiosis.

Chemokines

- Studies in human intestinal epithelial cells and jejunal biopsies show that CCL-5, CXCL-8 and CXCL-10 are upregulated in cryptosporidial infection and CXCL-8 is detectable in stool samples of infected humans.

Cytokines

- IFN- γ -independent pathways, including IL-15, play an important role in innate immune responses by activating natural killer cells.

Prostaglandins & substance P

- Cryptosporidial infection upregulates prostaglandin-E2 and -F2 production in human intestinal cells *in vitro*, which may contribute to antimicrobial peptide production, and downregulation of inflammatory cytokines.
- Substance P levels in jejunal biopsies from AIDS patients are higher in those with symptomatic cryptosporidiosis.

Adaptive immune responses

Cell-mediated immunity & T-cell responses

- T-cell immune responses play a critical role in resolution of cryptosporidial infection, predominantly via CD4⁺ cells and IFN- γ -mediated pathways.
- IFN- γ may play a role in T-cell memory responses.

Humoral immunity & antibody responses

- Several studies have reported serum antibody responses to cryptosporidial antigens following infection.
- Human volunteer studies show that those with pre-existing anticryptosporidial antibodies are protected from diarrheal symptoms but not infection.

***Cryptosporidium* antigens**

- A number of antigens, including gp900, p23/27, gp40 and gp15/17, that mediate *Cryptosporidium* host–parasite interactions induce antibody and or/ cell-mediated immune responses in humans.

Future perspective

- Investigation of genetic polymorphisms in Toll-like receptors and MBL genes and susceptibility to cryptosporidiosis in vulnerable populations.
- Investigation of the role of antimicrobial peptides in infected humans.

- Investigation of systemic and mucosal cell-mediated and humoral responses to putative protective antigens in longitudinal birth cohort studies in vulnerable human populations such as malnourished children in developing countries.
- Further studies in humans to determine whether antibody responses are themselves protective, or if they are just markers of cellular immune responses.
- Investigation of immune responses to glycotopes of putative protective antigens.
- Use of genomic and proteomic approaches to identify additional putative protective antigens.
- Development of targeted prophylactic and therapeutic immune-based strategies.

Acknowledgments

Financial & competing interests disclosure

Work in Honorine Ward's laboratory is supported by the NIH. Anoli Borad was supported by a fellowship from NIH T32 training grant AI07438. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Bibliography

Papers of special note have been highlighted as:

- of interest

1. Pierce KK, Kirkpatrick BD. Update on human infections caused by intestinal protozoa. *Curr Opin Gastroenterol* 2009;25(1):12–17. [PubMed: 19119509]
2. Dillingham RA, Lima AA, Guerrant RL. Cryptosporidiosis: epidemiology and impact. *Microbes Infect* 2002;4(10):1059–1066. [PubMed: 12191656]
3. Tzipori S, Ward H. Cryptosporidiosis: biology, pathogenesis and disease. *Microbes Infect* 2002;4(10):1047–1058. [PubMed: 12191655]
4. Huang DB, White AC. An updated review on *Cryptosporidium* and *Giardia*. *Gastroenterol Clin North Am* 2006;35(2):291–314. [PubMed: 16880067]
5. Caccio SM, Pozio E. Advances in the epidemiology, diagnosis and treatment of cryptosporidiosis. *Expert Rev Anti Infect Ther* 2006;4(3):429–443. [PubMed: 16771620]
6. Thompson RC, Olson ME, Zhu G, Enomoto S, Abrahamsen MS, Hijjawi NS. *Cryptosporidium* and cryptosporidiosis. *Adv Parasitol* 2005;59:77–158. [PubMed: 16182865]
7. Chappell CL, Okhuysen PC. Cryptosporidiosis. *Curr Opin Infect Dis* 2002;15(5):523–527. [PubMed: 12686887]
8. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. *Exp Parasitol* 2010;124(1):80–89. [PubMed: 19358845]
9. Wanyiri J, Ward H. Molecular basis of *Cryptosporidium*–host cell interactions: recent advances and future prospects. *Future Microbiol* 2006;1:201–208. [PubMed: 17661665] ▪ Reviews the current state of knowledge of *Cryptosporidium* antigens believed to mediate attachment and invasion of host cells.
10. Leav BA, Mackay M, Ward HD. *Cryptosporidium* species: new insights and old challenges. *Clin Infect Dis* 2003;36(7):903–908. [PubMed: 12652391]
11. Chen XM, Keithly JS, Paya CV, LaRusso NF. Cryptosporidiosis. *N Engl J Med* 2002;346(22):1723–1731. [PubMed: 12037153]

12. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection and identification. *Int J Parasitol* 2000;30(12–13):1305–1322. [PubMed: 11113257]
13. Rotz LD, Khan AS, Lillibridge SR, Ostroff SM, Hughes JM. Public health assessment of potential biological terrorism agents. *Emerg Infect Dis* 2002;8(2):225–230. [PubMed: 11897082]
14. Robinson P, Okhuysen PC, Chappell CL, et al. Substance p expression correlates with severity of diarrhea in cryptosporidiosis. *J Infect Dis* 2003;188(2):290–296. [PubMed: 12854086]
15. Laurent F, Kagnoff MF, Savidge TC, Naciri M, Eckmann L. Human intestinal epithelial cells respond to *Cryptosporidium parvum* infection with increased prostaglandin H synthase 2 expression and prostaglandin E2 and F2 α production. *Infect Immun* 1998;66(4):1787–1790. [PubMed: 9529115]
16. Chen XM, Levine SA, Splinter PL, et al. *Cryptosporidium parvum* activates nuclear factor κ B in biliary epithelia preventing epithelial cell apoptosis. *Gastroenterology* 2001;120(7):1774–1783. [PubMed: 11375958]
17. Ajjampur SSR, Rajendran P, Banerjee I, et al. Closing the diagnostic gap in diarrhoea in Indian children by the application of molecular techniques. *J Med Microbiol* 2008;57:1364–1368. [PubMed: 18927413]
18. Keusch GT. The history of nutrition: malnutrition, infection and immunity. *J Nutr* 2003;133(1):S336–S340.
19. Kirkpatrick BD, Haque R, Duggal P, et al. Association between *Cryptosporidium* infection and human leukocyte antigen class I and class II alleles. *J Infect Dis* 2008;197(3):474–478. [PubMed: 18248305]
20. Maggi P, Larocca AM, Quarto M, et al. Effect of antiretroviral therapy on cryptosporidiosis and microsporidiosis in patients infected with human immunodeficiency virus type 1. *Eur J Clin Microbiol Infect Dis* 2000;19(3):213–217. [PubMed: 10795595]
21. Nannini EC, Okhuysen PC. HIV1 and the gut in the era of highly active antiretroviral therapy. *Curr Gastroenterol Rep* 2002;4(5):392–398. [PubMed: 12228041]
22. Abubakar I, Aliyu SH, Arumugam C, Usman NK, Hunter PR. Treatment of cryptosporidiosis in immunocompromised individuals: systematic review and meta-analysis. *Br J Clin Pharmacol* 2007;63(4):387–393. [PubMed: 17335543]
23. Abubakar I, Aliyu SH, Arumugam C, Hunter PR, Usman NK. Prevention and treatment of cryptosporidiosis in immunocompromised patients. *Cochrane Database Syst Rev* 2007;1:CD004932. [PubMed: 17253532]
24. Theodos CM. Innate and cell-mediated immune responses to *Cryptosporidium parvum*. *Adv Parasitol* 1998;40:87–119. [PubMed: 9554071]
25. Riggs MW. Recent advances in cryptosporidiosis: the immune response. *Microbes Infect* 2002;4(10):1067–1080. [PubMed: 12191657]
26. Pantenburg B, Dann SM, Wang HC, et al. Intestinal immune response to human *Cryptosporidium* sp. infection. *Infect Immun* 2008;76(1):23–29. [PubMed: 17967863] • Reviews recent advances in the understanding of human intestinal immune responses in cryptosporidial infection.
27. Akira S. Toll-like receptors and innate immunity. *Adv Immunol* 2001;78:1–56. [PubMed: 11432202]
28. Medzhitov R. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001;1(2):135–145. [PubMed: 11905821]
29. Adachi O, Kawai T, Takeda K, et al. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* 1998;9(1):143–150. [PubMed: 9697844]
30. Akira S. Bacterial infections and Toll-like receptors. *Kekkaku* 2001;76(8):593–600. [PubMed: 11558139]
31. Chen XM, O'Hara SP, Nelson JB, et al. Multiple TLRs are expressed in human cholangiocytes and mediate host epithelial defense responses to *Cryptosporidium parvum* via activation of NF- κ B. *J Immunol* 2005;175(11):7447–7456. [PubMed: 16301652] • Reports recruitment of Toll-like receptor (TLR)-2 and TLR-4 to the site of infection in a human cholangiocyte model of infection *in vitro* and indicates that TLR-mediated pathways mediate innate immune responses to *Cryptosporidium parvum* by activation of NF- κ B.

32. Chen XM, Splinter PL, O'Hara SP, LaRusso NF. A cellular micro-RNA, let-7i, regulates Toll-like receptor 4 expression and contributes to cholangiocyte immune responses against *Cryptosporidium parvum* infection. *J Biol Chem* 2007;282(39):28929–28938. [PubMed: 17660297]
33. Priest JW, Mehlert A, Arrowood MJ, Riggs MW, Ferguson MA. Characterization of a low molecular weight glycolipid antigen from *Cryptosporidium parvum*. *J Biol Chem* 2003;278(52):52212–52222. [PubMed: 14557271]
34. Priest JW, Mehlert A, Moss DM, Arrowood MJ, Ferguson MA. Characterization of the glycosylphosphatidylinositol anchor of the immunodominant *Cryptosporidium parvum* 17-kDa antigen. *Mol Biochem Parasitol* 2006;149(1):108–112. [PubMed: 16759714]
35. Krishnegowda G, Hajjar AM, Zhu J, et al. Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity. *J Biol Chem* 2005;280(9):8606–8616. [PubMed: 15623512]
36. Wehkamp J, Schaubert J, Stange EF. Defensins and cathelicidins in gastrointestinal infections. *Curr Opin Gastroenterol* 2007;23(1):32–38. [PubMed: 17133082]
37. Zaalouk TK, Bajaj-Elliott M, George JT, McDonald V. Differential regulation of β -defensin gene expression during *Cryptosporidium parvum* infection. *Infect Immun* 2004;72(5):2772–2779. [PubMed: 15102787] • Describes defensin production in response to *C. parvum* infection of human intestinal epithelial cells and demonstrates that human β -defensins 1 and 2 have antimicrobial activity against *C. parvum in vitro*.
38. Takahashi K, Ip WE, Michelow IC, Ezekowitz RA. The mannose-binding lectin: a prototypic pattern recognition molecule. *Curr Opin Immunol* 2006;18(1):16–23. [PubMed: 16368230]
39. Kelly P, Jack DL, Naeem A, et al. Mannose-binding lectin is a component of innate mucosal defense against *Cryptosporidium parvum* in AIDS. *Gastroenterology* 2000;119(5):1236–1242. [PubMed: 11054381]
40. Kirkpatrick BD, Huston CD, Wagner D, et al. Serum mannose-binding lectin deficiency is associated with cryptosporidiosis in young Haitian children. *Clin Infect Dis* 2006;43(3):289–294. [PubMed: 16804841] • Reports that low levels of serum mannose-binding lectin are associated with increased susceptibility to cryptosporidiosis in children in Haiti.
41. Carmolli M, Duggal P, Haque R, et al. Deficient serum mannose-binding lectin levels and MBL2 polymorphisms increase the risk of single and recurrent *Cryptosporidium* infections in young children. *J Infect Dis* 2009;200(10):1540–1547. [PubMed: 19827946]
42. Maillot C, Gargala G, Delaunay A, et al. *Cryptosporidium parvum* infection stimulates the secretion of TGF- β , IL-8 and RANTES by Caco-2 cell line. *Parasitol Res* 2000;86(12):947–949. [PubMed: 11133108]
43. Laurent F, Eckmann L, Savidge TC, et al. *Cryptosporidium parvum* infection of human intestinal epithelial cells induces the polarized secretion of C-X-C chemokines. *Infect Immun* 1997;65(12):5067–5073. [PubMed: 9393797]
44. Kirkpatrick BD, Noel F, Rouzier PD, et al. Childhood cryptosporidiosis is associated with a persistent systemic inflammatory response. *Clin Infect Dis* 2006;43(5):604–608. [PubMed: 16886154]
45. Alcantara CS, Yang CH, Steiner TS, et al. Interleukin-8, tumor necrosis factor- α , and lactoferrin in immunocompetent hosts with experimental and Brazilian children with acquired cryptosporidiosis. *Am J Trop Med Hyg* 2003;68(3):325–328. [PubMed: 12685639]
46. Kirkpatrick BD, Daniels MM, Jean SS, et al. Cryptosporidiosis stimulates an inflammatory intestinal response in malnourished Haitian children. *J Infect Dis* 2002;186(1):94–101. [PubMed: 12089667]
47. Wang HC, Dann SM, Okhuysen PC, et al. High levels of CXCL10 are produced by intestinal epithelial cells in AIDS patients with active cryptosporidiosis but not after reconstitution of immunity. *Infect Immun* 2007;75(1):481–487. [PubMed: 17043107] • Describes CXCL10 production in intestinal tissues in response to acute *Cryptosporidium* infection in AIDS patients and reversal following reconstitution of immune responses.
48. Lean IS, McDonald V, Pollok RC. The role of cytokines in the pathogenesis of *Cryptosporidium* infection. *Curr Opin Infect Dis* 2002;15(3):229–234. [PubMed: 12015455]

49. Pollok RC, Farthing MJ, Bajaj-Elliott M, Sanderson IR, McDonald V. Interferon γ induces enterocyte resistance against infection by the intracellular pathogen *Cryptosporidium parvum*. *Gastroenterology* 2001;120(1):99–107. [PubMed: 11208718]
50. Choudhry N, Korbel DS, Edwards LA, Bajaj-Elliott M, McDonald V. Dysregulation of interferon- γ -mediated signalling pathway in intestinal epithelial cells by *Cryptosporidium parvum* infection. *Cell Microbiol* 2009;11(9):1354–1364. [PubMed: 19473199]
51. Gomez Morales MA, Ausiello CM, Guarino A, et al. Severe, protracted intestinal cryptosporidiosis associated with interferon γ deficiency: pediatric case report. *Clin Infect Dis* 1996;22(5):848–850. [PubMed: 8722945]
52. White AC, Robinson P, Okhuysen PC, et al. Interferon- γ expression in jejunal biopsies in experimental human cryptosporidiosis correlates with prior sensitization and control of oocyst excretion. *J Infect Dis* 2000;181(2):701–709. [PubMed: 10669358]
53. Robinson P, Okhuysen PC, Chappell CL, et al. Expression of IL-15 and IL-4 in IFN- γ -independent control of experimental human *Cryptosporidium parvum* infection. *Cytokine* 2001;15(1):39–46. [PubMed: 11509007]
54. Dann SM, Wang HC, Gambarin KJ, et al. Interleukin-15 activates human natural killer cells to clear the intestinal protozoan *Cryptosporidium*. *J Infect Dis* 2005;192(7):1294–1302. [PubMed: 16136475] • Describes activation of peripheral blood natural killer cells by IL-15 in humans and demonstrates their ability to lyse *Cryptosporidium*-infected intestinal epithelial cells.
55. Robinson P, Okhuysen PC, Chappell CL, et al. Transforming growth factor b1 is expressed in the jejunum after experimental *Cryptosporidium parvum* infection in humans. *Infect Immun* 2000;68(9):5405–5407. [PubMed: 10948171]
56. Robinson P, Okhuysen PC, Chappell CL, et al. Expression of tumor necrosis factor α and interleukin 1 β in jejuna of volunteers after experimental challenge with *Cryptosporidium parvum* correlates with exposure but not with symptoms. *Infect Immun* 2001;69(2):1172–1174. [PubMed: 11160015]
57. Mazumdar S, Das KM. Immunocytochemical localization of vasoactive intestinal peptide and substance P in the colon from normal subjects and patients with inflammatory bowel disease. *Am J Gastroenterol* 1992;87(2):176–181. [PubMed: 1370872]
58. McDonald V. Host cell-mediated responses to infection with *Cryptosporidium*. *Parasite Immunol* 2000;22(12):597–604. [PubMed: 11123751]
59. Gomez Morales MA, Pozio E. Humoral and cellular immunity against *Cryptosporidium* infection. *Curr Drug Targets Immune Endocr Metabol Disord* 2002;2(3):291–301. [PubMed: 12476493]
60. Pozio E, Rezza G, Boschini A, et al. Clinical cryptosporidiosis and human immunodeficiency virus (HIV)-induced immunosuppression: findings from a longitudinal study of HIV-positive and HIV-negative former injection drug users. *J Infect Dis* 1997;176(4):969–975. [PubMed: 9333155]
61. Schmidt W, Wahnschaffe U, Schafer M, et al. Rapid increase of mucosal CD4 T cells followed by clearance of intestinal cryptosporidiosis in an AIDS patient receiving highly active antiretroviral therapy. *Gastroenterology* 2001;120(4):984–987. [PubMed: 11231952]
62. Gomez Morales MA, Ausiello CM, Urbani F, Pozio E. Crude extract and recombinant protein of *Cryptosporidium parvum* oocysts induce proliferation of human peripheral blood mononuclear cells *in vitro*. *J Infect Dis* 1995;172(1):211–216. [PubMed: 7797913]
63. Gomez Morales MA, La Rosa G, Ludovisi A, Onori AM, Pozio E. Cytokine profile induced by *Cryptosporidium* antigen in peripheral blood mononuclear cells from immunocompetent and immunosuppressed persons with cryptosporidiosis. *J Infect Dis* 1999;179(4):967–973. [PubMed: 10068593]
64. Gomez Morales MA, Mele R, Ludovisi A. *Cryptosporidium parvum*-specific CD4 Th1 cells from sensitized donors responding to both fractionated and recombinant antigenic proteins. *Infect Immun* 2004;72(3):1306–1310. [PubMed: 14977932] • Describes isolation of T-cell clones from peripheral blood mononuclear cells of healthy individuals previously exposed to cryptosporidiosis in response to stimulation with native and recombinant antigens.
65. Smith LM, Priest JW, Lammie PJ, Mead JR. Human T and B cell immunoreactivity to a recombinant 23-kDa *Cryptosporidium parvum* antigen. *J Parasitol* 2001;87(3):704–707. [PubMed: 11426740]

66. Preidis GA, Wang HC, Lewis DE, et al. Seropositive human subjects produce interferon γ after stimulation with recombinant *Cryptosporidium hominis* gp15. *Am J Trop Med Hyg* 2007;(3):77. 583–585. • Describes production of IFN- γ by peripheral blood mononuclear cells of seropositive but not seronegative individuals in response to *ex vivo* stimulation with recombinant *Cryptosporidium hominis* gp15.
67. Winkelstein JA, Marino MC, Ochs H, et al. The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. *Medicine (Baltimore)* 2003;82(6):373–384. [PubMed: 14663287]
68. Wolska-Kusnierz B, Bajer A, Caccio S, et al. *Cryptosporidium* infection in patients with primary immunodeficiencies. *J Pediatr Gastroenterol Nutr* 2007;45(4):458–464. [PubMed: 18030213]
69. Ungar BL, Soave R, Fayer R, Nash TE. Enzyme immunoassay detection of immunoglobulin M and G antibodies to *Cryptosporidium* in immunocompetent and immunocompromised persons. *J Infect Dis* 1986;153(3):570–578. [PubMed: 3950440]
70. Dann SM, Okhuysen PC, Salameh BM, DuPont HL, Chappell CL. Fecal antibodies to *Cryptosporidium parvum* in healthy volunteers. *Infect Immun* 2000;68(9):5068–5074. [PubMed: 10948127]
71. Chappell CL, Okhuysen PC, Sterling CR, Wang C, Jakubowski W, Dupont HL. Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. *Am J Trop Med Hyg* 1999;60(1):157–164. [PubMed: 9988341]
72. Moss DM, Chappell CL, Okhuysen PC, et al. The antibody response to 27-, 17-, and 15-kDa *Cryptosporidium* antigens following experimental infection in humans. *J Infect Dis* 1998;178(3): 827–833. [PubMed: 9728553]
73. Frost FJ, Tollestrup K, Craun GF, Fairley CK, Sinclair MI, Kunde TR. Protective immunity associated with a strong serological response to a *Cryptosporidium*-specific antigen group, in HIV-infected individuals. *J Infect Dis* 2005;192(4):618–621. [PubMed: 16028130]
74. Frost FJ, Roberts M, Kunde TR, et al. How clean must our drinking water be: the importance of protective immunity. *J Infect Dis* 2005;191(5):809–814. [PubMed: 15688300] • Reports that individuals who consume surface-derived drinking water are more likely to display a strong antibody response to 15–17-kDa *Cryptosporidium* antigens and to be at lower risk of acquiring cryptosporidial infection.
75. O'Connor RM, Burns PB, Ha-Ngoc T, et al. Polymorphic mucin antigens CpMuc4 and CpMuc5 are integral to *Cryptosporidium parvum* infection *in vitro*. *Eukaryot Cell* 2009;8(4):461–469. [PubMed: 19168754]
76. Xu P, Widmer G, Wang Y, et al. The genome of *Cryptosporidium hominis*. *Nature* 2004;431(7012):1107–1112. [PubMed: 15510150]
77. Abrahamsen MS, Templeton TJ, Enomoto S, et al. Complete genome sequence of the apicomplexan, *Cryptosporidium parvum*. *Science* 2004;304(5669):441–445. [PubMed: 15044751]
78. Smith HV, Nichols RA, Grimason AM. *Cryptosporidium* excystation and invasion: getting to the guts of the matter. *Trends Parasitol* 2005;21(3):133–142. [PubMed: 15734661]
79. Okhuysen PC, Chappell CL. *Cryptosporidium* virulence determinants – are we there yet? *Int J Parasitol* 2002;32(5):517–525. [PubMed: 11943224]
80. Cevallos AM, Bhat N, Verdon R, et al. Mediation of *Cryptosporidium parvum* infection *in vitro* by mucin-like glycoproteins defined by a neutralizing monoclonal antibody. *Infect Immun* 2000;68(9):5167–5175. [PubMed: 10948140]
81. Priest JW, Kwon JP, Arrowood MJ, Lammie PJ. Cloning of the immunodominant 17-kDa antigen from *Cryptosporidium parvum*. *Mol Biochem Parasitol* 2000;106(2):261–271. [PubMed: 10699255]
82. Strong WB, Gut J, Nelson RG. Cloning and sequence analysis of a highly polymorphic *Cryptosporidium parvum* gene encoding a 60-kilodalton glycoprotein and characterization of its 15- and 45-kilodalton zoite surface antigen products. *Infect Immun* 2000;68(7):4117–4134. [PubMed: 10858229]
83. Winter G, Gooley AA, Williams KL, Slade MB. Characterization of a major sporozoite surface glycoprotein of *Cryptosporidium parvum*. *Funct Integr Genomics* 2000;1(3):207–217. [PubMed: 11793239]

84. Wanyiri JW, O'Connor R, Allison G, et al. Proteolytic processing of the *Cryptosporidium* glycoprotein gp40/15 by human furin and by a parasite-derived furin-like protease activity. *Infect Immun* 2007;75(1):184–192. [PubMed: 17043102]
85. Wanyiri JW, Techasintana P, O'Connor RM, Blackman MJ, Kim K, Ward HD. Role of CpSUB1, a subtilisin-like protease, in *Cryptosporidium parvum* infection *in vitro*. *Eukaryot Cell* 2009;8(4):470–477. [PubMed: 19168760]
86. O'Connor RM, Wanyiri JW, Cevallos AM, Priest JW, Ward HD. *Cryptosporidium parvum* glycoprotein gp40 localizes to the sporozoite surface by association with gp15. *Mol Biochem Parasitol* 2007;156(1):80–83. [PubMed: 17719100]
87. Gut J, Nelson RG. *Cryptosporidium parvum* sporozoites deposit trails of 11A5 antigen during gliding locomotion and shed 11A5 antigen during invasion of MDCK cells *in vitro*. *J Eukaryot Microbiol* 1994;41(5):S42–S43.
88. Cevallos AM, Zhang X, Waldor MK, et al. Molecular cloning and expression of a gene encoding *Cryptosporidium parvum* glycoproteins gp40 and gp15. *Infect Immun* 2000;68(7):4108–4116. [PubMed: 10858228]
89. Priest JW, Bern C, Xiao L, et al. Longitudinal analysis of *Cryptosporidium* species-specific immunoglobulin G antibody responses in Peruvian children. *Clin Vaccine Immunol* 2006;13(1):123–131. [PubMed: 16426009] • Describes longitudinal serum antibody responses to 17- and 27-kDa antigens in Peruvian children with cryptosporidiosis.
90. Khan WA, Rogers KA, Karim MM, et al. Cryptosporidiosis among Bangladeshi children with diarrhea: a prospective, matched, case–control study of clinical features, epidemiology and systemic antibody responses. *Am J Trop Med Hyg* 2004;71(4):412–419. [PubMed: 15516636]
91. Allison, G.; Rogers, K.; Khan, W., et al. Systemic IgG response to recombinant *Cryptosporidium hominis* and *Cryptosporidium parvum* gp40 and gp15 antigens in Bangladeshi children with diarrhea. Presented at: 54th Annual Meeting of the American Society of Tropical Medicine and Hygiene; Washington, DC, USA. December 2005; p. 11-15.
92. O'Connor RM, Wanyiri JW, Cevallos AM, Priest JW, Ward HD. *Cryptosporidium parvum* glycoprotein gp40 localizes to the sporozoite surface by association with gp15. *Mol Biochem Parasitol* 2007;156(1):80–83. [PubMed: 17719100]
93. Ajajampur SS, Gladstone BP, Selvapandian D, Muliyl JP, Ward H, Kang G. Molecular and spatial epidemiology of cryptosporidiosis in children in a semiurban community in South India. *J Clin Microbiol* 2007;45(3):915–920. [PubMed: 17251402]
94. Ajajampur, SR.; Banda, K.; Allison, G.; Ward, H.; Kang, G. Antibody response to cryptosporidial antigens gp40 and gp15 in children in Southern India. Presented at: Annual Meeting of the Infectious Diseases Society of America; Toronto, Canada. October 2006; p. 12-15.
95. Perryman LE, Jasmer DP, Riggs MW, Bohnet SG, McGuire TC, Arrowood MJ. A cloned gene of *Cryptosporidium parvum* encodes neutralization-sensitive epitopes. *Mol Biochem Parasitol* 1996;80(2):137–147. [PubMed: 8892291]
96. Borad, AJ.; Allison, G.; Khan, WA., et al. Systemic antibody responses to *Cryptosporidium parvum* Cp27 in Bangladeshi children with diarrhea and cryptosporidiosis. Presented at: Annual Immunoparasitology Meeting; Woodshole, MA, USA. April–1 May 2007; p. 26
97. Sturbaum GD, Jost BH, Sterling CR. Nucleotide changes within three *Cryptosporidium parvum* surface protein encoding genes differentiate genotype I from genotype II isolates. *Mol Biochem Parasitol* 2003;128(1):87–90. [PubMed: 12706801]
98. Uhl EW, O'Connor RM, Perryman LE, Riggs MW. Neutralization-sensitive epitopes are conserved among geographically diverse isolates of *Cryptosporidium parvum*. *Infect Immun* 1992;60(4):1703–1706. [PubMed: 1372299]
99. Petersen C, Gut J, Doyle PS, Crabb JH, Nelson RG, Leech JH. Characterization of a > 900,000-M(r) *Cryptosporidium parvum* sporozoite glycoprotein recognized by protective hyperimmune bovine colostrum immunoglobulin. *Infect Immun* 1992;60(12):5132–5138. [PubMed: 1452347]
100. Barnes DA, Bonnin A, Huang JX, et al. A novel multi-domain mucin-like glycoprotein of *Cryptosporidium parvum* mediates invasion. *Mol Biochem Parasitol* 1998;96(1–2):93–110. [PubMed: 9851610]

101. Ward H, Cevallos AM. *Cryptosporidium*: molecular basis of host–parasite interaction. *Adv Parasitol* 1998;40:151–185. [PubMed: 9554073]
102. Langhorst J, Junge A, Rueffer A, et al. Elevated human β -defensin-2 levels indicate an activation of the innate immune system in patients with irritable bowel syndrome. *Am J Gastroenterol* 2009;104(2):404–410. [PubMed: 19174795]
103. O'Connor RM, Kim K, Khan F, Ward HD. Expression of Cpgp40/15 in *Toxoplasma gondii*: a surrogate system for the study of *Cryptosporidium* glycoprotein antigens. *Infect Immun* 2003;71(10):6027–6034. [PubMed: 14500524]
104. Robin G, Fraser D, Orr N, et al. *Cryptosporidium* infection in Bedouin infants assessed by prospective evaluation of anticryptosporidial antibodies and stool examination. *Am J Epidemiol* 2001;153(2):194–201. [PubMed: 11159166]

Table 1Recent studies of innate immune responses to *Cryptosporidium* in humans.

Type of study	Findings	Ref.
<i>Mannose-binding lectin</i>		
Cross-sectional study in AIDS patients in Zambia	Increased risk for cryptosporidiosis in patients homozygous for <i>mbl2</i> structural gene mutations	[39]
Case-control study of children in Haiti	Serum mannose-binding lectin deficiency was significantly associated with cryptosporidiosis	[40]
Prospective cohort study of children in Bangladesh	Serum mannose-binding lectin deficiency and <i>mbl2</i> polymorphisms were significantly associated with cryptosporidiosis	[41]
<i>Chemokines and cytokines</i>		
Study of experimentally infected human volunteers	TGF- β was expressed in jejunal biopsies of some volunteers shedding oocysts	[55]
Study of experimentally infected human volunteers	TNF- α and IL-1- β were expressed in jejunal biopsies of some volunteers but were not associated with symptoms	[56]
Study of experimentally infected human volunteers	IL-15 was expressed in jejunal biopsies of volunteers who did not express IFN- γ and was associated with lower oocyst shedding. IL-4 expression was not associated with symptoms	[53]
Prospective triple cohort study in children in Haiti	Levels of IL-8, TNF- α , IL-13 and IL-4 but not IFN- γ were elevated in stool samples of cases with cryptosporidiosis	[46]
Study of experimentally infected human volunteers and children in Brazil	IL-8 was detectable in stool samples of some patients with cryptosporidiosis	[45]
Prospective case-control cohort study of children in Haiti	Peripheral blood mononuclear cells of children with cryptosporidiosis stimulated <i>ex vivo</i> with <i>Cryptosporidium</i> antigens expressed higher levels of CXCL-8 and TNF- α compared to controls	[44]
Case-control study in AIDS patients	Higher levels of CXCL-10 were detected in jejunal biopsies of AIDS patients with cryptosporidiosis compared with controls	[47]
<i>Substance P</i>		
Study of experimentally infected human volunteers and AIDS patients	Substance P mRNA and protein expression were higher in jejunal biopsies of symptomatic volunteers and AIDS patients compared with asymptomatic volunteers	[14]

Table 2

Recent studies of cell-mediated immune responses to *Cryptosporidium* in humans.

Type of study	Findings	Ref.
Study of immunocompetent individuals and AIDS patients	IFN- γ was produced from peripheral blood mononuclear cells stimulated <i>ex vivo</i> with crude cryptosporidial extract in patients with cryptosporidiosis	[63]
Study of experimentally infected human volunteers and AIDS patients	IFN- γ mRNA was detected in jejunal biopsies of volunteers after infection but not in AIDS patients with cryptosporidiosis. IFN- γ expression correlated significantly with the presence of anticryptosporidial antibodies and absence of oocyst shedding	[52]
Observational study in patients from Haiti	Patients with prior cryptosporidiosis displayed antibody- and cell-mediated responses to Cp23 and crude cryptosporidial antigens. Antibody responses were higher in those with T-cell responses	[65]
Study of immunocompetent patients	T-cell clones derived from peripheral blood mononuclear cells stimulated <i>ex vivo</i> with native and recombinant cryptosporidial antigens were TCR- $\alpha\beta^+$, CD4 $^+$, CD45RO $^+$ and expressed either a Th1 (IFN- γ) or Th0 (IL-4 and IL-5) profile	[64]
Study of seropositive and seronegative human volunteers	CD4 $^+$ and CD8 $^+$ cells from seropositive donors produced greater amounts of IFN- γ than seronegative donors in response to <i>ex vivo</i> stimulation with recombinant gp15	[66]

Table 3

Recent studies of humoral immune responses to *Cryptosporidium* in humans.

Type of study	Findings	Ref.
Study of experimentally infected human volunteers	Volunteers with pre-existing anticryptosporidial antibodies had less diarrhea and oocyst shedding upon challenge with low doses of oocysts.	[71]
Study of experimentally infected human volunteers	Anticryptosporidial fecal IgA was detectable during active cryptosporidial infection.	[70]
Longitudinal study of Bedouin children in Israel	Serum anti- <i>Cryptosporidium parvum</i> IgG levels were high soon after birth, dropped by 6 months of age, and continuously increased until 23 months of age.	[104]
Case-control study of children with diarrhea in Bangladesh	Serum IgM levels to <i>C. parvum</i> antigens were higher in cases with <i>Cryptosporidium</i> infection than uninfected controls. Increase in IgG levels over 3 weeks was greater in cases compared with controls. Persistent diarrhea was associated with lower IgA and IgM levels.	[90]
Cross-sectional study of HIV-infected patients in Australia	Strong antibody responses to 27-kDa antigen were associated with reduced risk of diarrhea without weight loss.	[73]
Observational study of people served by ground and surface water sources	Strong antibody response to 15/17-kDa antigen were associated with reduced risk of diarrhea in users of surface water sources.	[74]
Longitudinal cohort study of children in Peru	Antibody responses to 15/17-kDa antigens were greater in older children, those with multiple infections and those with asymptomatic infection.	[89]