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Glycoproteins and Gal-GalNAc cause *Cryptosporidium* to switch from an invasive sporozoite to a replicative trophozoite

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Abstract

The apicomplexan parasite *Cryptosporidium* causes cryptosporidiosis, a diarrheal disease that can become chronic and life threatening in immunocompromised and malnourished people. There is no effective drug treatment for those most at risk of severe cryptosporidiosis. The disease pathology is due to a repeated cycle of host cell invasion and parasite replication that amplifies parasite numbers and destroys the intestinal epithelium. This study aimed to better understand the *Cryptosporidium* replication cycle by identifying molecules that trigger the switch from invasive sporozoite to replicative trophozoite. Our approach was to treat sporozoites of *Cryptosporidium parvum* and *Cryptosporidium hominis*, the species causing most human cryptosporidiosis, with various media under axenic conditions and examine the parasites for rounding and nuclear division as markers of trophozoite development and replication, respectively. FBS had a concentration-dependent effect on trophozoite development in both species. Trophozoite development in *C. parvum*, but not *C. hominis*, was enhanced when RPMI supplemented with 10% FBS (RPMI-FBS) was conditioned by HCT-8 cells for 3 h. The effect of non-conditioned and HCT-8 conditioned RPMI-FBS on trophozoite development was abrogated by proteinase K and sodium metaperiodate pretreatment, indicating a glycoprotein trigger. *Cryptosporidium parvum* and *C. hominis* trophozoite development also was triggered by Gal-GalNAc in a concentration-dependent manner. *Cryptosporidium parvum* replication was greatest following treatments with Gal-GalNAc, followed by conditioned RPMI-FBS and non-conditioned RPMI-FBS ($P < 0.05$). *Cryptosporidium hominis* replication was significantly less than that in *C. parvum* for all treatments ($P < 0.05$), and was greatest at the highest tested concentration of Gal-GalNAc (1 mM).

Graphical Abstract

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Keywords

Cryptosporidium; Asexual replication; Trophozoite; Sporozoite; Glycan; Glycoprotein; HCT-8 cells

1. Introduction

The apicomplexan parasite *Cryptosporidium* infects all major vertebrate groups and causes the diarrheal disease cryptosporidiosis. Human cryptosporidiosis, caused primarily by *Cryptosporidium parvum* and *Cryptosporidium hominis*, is associated with severe illness and toddler mortality in developing countries (Kotloff et al., 2013) and chronic, life-threatening disease in immunodeficient and malnourished adults (Garrido Davila and Ramirez Ronda, 1991; Solorzano-Santos et al., 2000; Costa et al., 2011). New drugs to treat cryptosporidiosis are urgently needed because current drugs lack efficacy, particularly in those with a compromised immune system (Striepen, 2013). One aspect of the infection with potential for drug development is the repeating cycle of host cell invasion and parasite replication that rapidly and massively amplifies *Cryptosporidium* numbers in the intestinal epithelium, resulting in the pathology that defines cryptosporidiosis. The task of identifying inhibitors of this cycle will be helped by a better understanding of how *Cryptosporidium* transitions from an invasive to a replicative mode.

Current knowledge of apicomplexan invasion and replication mechanisms is derived primarily from studies on *Toxoplasma gondii*. During invasion, the anterior surface of the *T. gondii* zoite forms a junction with the host cell membrane through the interaction of rhoptry neck proteins (RONs), which are exported from the parasite to the host cell membrane, and the parasite-associated apical membrane antigen 1 (AMA1) (Besteiro et al., 2011; Lamarque et al., 2011). Powered by an actomyosin motor, the zoite moves the junction posteriorly along its surface, moving it through the host cell membrane and into a cytoplasmic vacuole (Tyler et al., 2011). AMA1 is cleaved by an intramembrane protease as the parasite enters

the vacuole (Buguliskis et al., 2010), and the cleaved cytoplasmic tail may trigger parasite replication (Santos et al., 2011), although this remains in some doubt (Parussini et al., 2012; Shen et al., 2014).

In contrast to *T. gondii*, *Cryptosporidium* invasion is a passive process that does not require actomyosin motility machinery (Forney et al., 1998) and results in an extracytoplasmic rather than cytoplasmic parasitophorous vacuole. In studies on *C. parvum*, it has been shown that sporozoite surface lectins bind to Gal-GalNAc ligands on host cells (Chen and Larusso, 2000; Bhat et al., 2007). Aggregation of Gal-GalNAc glycoproteins in the host cell membrane at the sporozoite attachment site initiates a signaling cascade that culminates in actin-dependent membrane protrusion and encapsulation of the sporozoite in an extracytoplasmic vacuole (Nelson et al., 2006; O'Hara et al., 2008). It is not known what causes the invasive *Cryptosporidium* sporozoite to transform into a replicative trophozoite, but its unusual invasion mechanism and unique extracytoplasmic niche in the host cell suggests a different trigger to that in *T. gondii*.

Cryptosporidium is more closely related to the gregarines, invertebrate parasites that frequently develop extracellular trophozoites, than to *T. gondii* and other apicomplexans that parasitize vertebrates (Barta and Thompson, 2006; Templeton et al., 2010; Cavalier-Smith, 2014). Consistent with its gregarine affinity, *Cryptosporidium* can occasionally develop extracellular trophozoites in cell culture (Rosales et al., 2005), and trophozoites and other life cycle stages have been observed even in the absence of host cells (Hijjawi et al., 2004, 2010; Boxell et al., 2008; Zhang et al., 2009; Koh et al., 2013; Yang et al., 2015). We reasoned that the trigger of trophozoite development under axenic conditions would be similar to that in host cells, and that axenic culture would provide a simple model to identify the trigger. Using this approach, here we show that banana-shaped *Cryptosporidium* sporozoites become rounded and undergo nuclear division, hallmarks of a transformation to replicative trophozoites, when triggered by Gal-GalNAc and by glycoproteins in FBS and the secretome of HCT-8 cells.

2. Materials and methods

2.1. Source and excystation of *Cryptosporidium* oocysts

A mouse passaged *C. parvum* Iowa isolate was purchased from Waterborne Inc. (New Orleans, LA, USA). *Cryptosporidium hominis* TU502 was propagated in a gnotobiotic pig at Tufts University, MA, USA (Tzipori et al., 1994). Animal experiments were approved by the Tufts University Animal Use and Care Committee, Massachusetts, USA. Oocysts of *C. parvum* and *C. hominis* were treated with 10% sodium hypochlorite for 10 min at 4°C, washed twice with PBS, and excysted for 1 h at 37°C and 5% CO₂ in RPMI-1640 supplemented with 0.8% sodium taurocholate (Sigma-Aldrich, St. Louis, MO, USA).

2.2. Preparation of RPMI with 10% FBS (RPMI-FBS) and HCT-8 conditioned RPMI-FBS

To prepare RPMI-FBS, RPMI-1640 was supplemented with 10% Opti-MEM, 10% FBS, antibiotic/antimycotic solution, 1 mM sodium pyruvate, 50 mM glucose, 0.2 mM ascorbic acid, 2.3 µM folic acid, 29 µM 4-aminobenzoic acid and 8.4 µM calcium pantothenate. RPMI-FBS was conditioned by incubation with confluent HCT-8 cells for 3 h at 37°C and

5% CO₂. Non-conditioned and HCT-8 conditioned RPMI-FBS were stored at 37°C and 5% CO₂ until use.

2.3. Treatment of sporozoites with non-conditioned and HCT-8 conditioned RPMI-FBS

For all treatments, excysted sporozoites of *C. parvum* and *C. hominis* were washed once with PBS, resuspended in 200 µL of media, and incubated at 37°C and 5% CO₂ for 0, 3, 4, 5 or 6 h. Treatments consisted of non-conditioned and conditioned RPMI-FBS; RPMI supplemented with 0, 5, 10 or 20% FBS; and non-conditioned and conditioned RPMI-FBS that was pretreated with proteinase K or sodium metaperiodate. Proteinase K (5 mg/ml) was added to RPMI-FBS and conditioned RPMI-FBS and incubated at 55°C overnight to digest proteins (Nichols et al., 2006). Proteinase K was subsequently inactivated by incubation at 90°C. Heat-inactivated proteinase K was added to non-conditioned and conditioned RPMI-FBS as a control. To oxidize glycans, an equal volume of 100 mM sodium metaperiodate (pH 7.2) was incubated with RPMI-FBS or conditioned RPMI-FBS overnight at 4°C in the dark. Excess sodium metaperiodate was removed by adding an equal volume of 260 mM glycerol. As a control, glycerol was added directly to non-conditioned or conditioned RPMI-FBS without sodium metaperiodate pretreatment.

Following treatments, zoites were spun onto a glass slide at 2,000 g for 5 min using a Shandon CytoSpin 2 (Thermo-Scientific, Waltham, MA, USA), fixed in 100% methanol for 15 min and washed twice with PBS for 3 min. Fixed zoites were blocked with 1% BSA for 20 min at room temperature, washed with PBS, and labeled with FITC- or TRITC-SporoGlo (Waterborne Inc. New Orleans, LA, USA). Slides were mounted and the numbers of banana-shaped and rounded zoites were determined under differential interference contrast (DIC) and fluorescence illumination in each of 20, randomly selected high-powered fields using an Olympus BX61 microscope.

2.4. Viability assessment

The viability of *C. parvum* and *C. hominis* zoites was determined following exposure to non-conditioned and conditioned RPMI-FBS for 3, 4, 5, 6, 7, 8 and 12 h. Zoites were incubated with 0.04 mM fluorescein diacetate (FDA) and 7 µM propidium iodide (PI) in PBS for 3 min at room temperature (Arrowood et al., 1991). A wet mount of stained zoites was prepared and the number of green fluorescing (FDA positive; viable) and red fluorescing (PI positive; non-viable) zoites was determined under fluorescence illumination in each of 20, randomly selected high-powered fields using an Olympus BX61 microscope.

2.5. Treatment of sporozoites with Gal-GalNAc

Sporozoites of *C. parvum* and *C. hominis* were treated with 0 or 10 µM Gal-GalNAc (Benzyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-galactopyranoside, product code B5019, Sigma-Aldrich) in PBS, non-conditioned RPMI-FBS or conditioned RPMI-FBS at 37°C and 5% CO₂ for 1 or 7 h. In a separate experiment, sporozoites of *C. parvum* and *C. hominis* were incubated with 0 µM, 10 µM, 100 µM or 1 mM Gal-GalNAc in PBS at 37°C and 5% CO₂ for 7 h. Slides were stained as described in Section 2.3, with additional staining of nuclei using 300 nM DAPI. The numbers of banana-shaped and rounded zoites and the proportion of zoites with two or more nuclei were determined under DIC and

fluorescence illumination in each of 20 randomly selected high-powered fields using an Olympus BX61 microscope.

2.6. Statistical analyses

The proportion of rounded, viable, or multinucleate zoites in each of 20 high-powered fields was used to calculate mean and S.D. values. Differences between treatments were determined at the 5% significance level ($P < 0.05$) using the Student's *t*-test with the Holm-Šídák correction for multiple comparisons (Holm, 1979). Statistical analyses were carried out using Prism 6 (Graphpad, La Jolla, CA, USA).

3. Results

3.1. Non-conditioned and HCT-8 conditioned RPMI-FBS cause *C. parvum* and *C. hominis* sporozoites to become rounded

For both species, banana-shaped sporozoites (mean length to width ratio: $2.96 \pm 0.54 \mu\text{m}$) became rounded (mean length to width ratio: $1.14 \pm 0.14 \mu\text{m}$) following treatments in non-conditioned and HCT-8 conditioned RPMI-FBS (Fig. 1). In non-conditioned RPMI-FBS, a greater proportion of *C. hominis* than *C. parvum* was rounded after 3, 4 and 5 h ($P < 0.05$), but there was no significant difference between the species after 6 h. Conditioning of RPMI-FBS with HCT-8 cells increased the proportion of *C. parvum* sporozoites that became rounded, particularly at early time points: $63.4 \pm 4.1\%$ and $94.2 \pm 4.5\%$ of *C. parvum* were rounded after 3 and 4 h treatments, respectively, in conditioned RPMI-FBS, compared with $6.9 \pm 1.9\%$ and $31.1 \pm 2.1\%$, respectively, in non-conditioned RPMI-FBS. Compared with *C. parvum*, conditioning of RPMI-FBS had little effect on *C. hominis* rounding, particularly at early time points.

The proportions of *C. parvum* and *C. hominis* that were rounded increased with the concentration of FBS in RPMI (Fig. 2). When FBS was excluded, only $12.2 \pm 2.0\%$ and $14.1 \pm 2.7\%$ of *C. parvum* and *C. hominis*, respectively, were rounded after 6 h, compared with $95.8 \pm 5.2\%$ and $91.9 \pm 8.4\%$, respectively, in RPMI with 20% FBS.

Collectively, these data show that molecules in FBS cause banana-shaped sporozoites of *C. parvum* and *C. hominis* to become rounded, and that molecules secreted by HCT-8 cells increase rounding in *C. parvum*.

3.2. *Cryptosporidium parvum* and *C. hominis* remain viable in non-conditioned and HCT-8 conditioned RPMI-FBS

Some studies on axenic *Cryptosporidium* development have used the change in sporozoite morphology from banana-shaped to rounded to indicate trophozoite development (Hijjawi et al., 2004, 2010; Boxell et al., 2008), while others have reported that rounding results from a loss of sporozoite viability (Petry et al., 2009; Matsubayashi et al., 2010). To assess the likelihood that rounding is degenerative rather than developmental, viability was examined after treatment of *C. parvum* and *C. hominis* sporozoites with non-conditioned or conditioned RPMI-FBS for periods ranging from 3 to 12 h (Fig. 3). In conditioned RPMI-FBS, 100% of *C. parvum* zoites were viable after 6 h, decreasing to $91.2 \pm 4.7\%$ after 12 h.

In non-conditioned RPMI-FBS, $98.1 \pm 3.7\%$ and $88.9 \pm 4.2\%$ of *C. parvum* were viable after 6 and 12 h, respectively. *Cryptosporidium hominis* was less viable than *C. parvum* at time points between 5 and 12 h in conditioned RPMI-FBS and between 5 and 7 h in non-conditioned RPMI-FBS ($P < 0.05$). In conditioned RPMI-FBS, the viability of *C. hominis* sporozoites decreased from $96.3 \pm 5.7\%$ after 3 h to $89.4 \pm 5.1\%$ after 6 h and $84.0 \pm 5.7\%$ after 12 h. Similar reductions in *C. hominis* viability were observed in non-conditioned RPMI-FBS. These relatively minor reductions in viability, particularly during the initial 6 h period when most cells became rounded, are evidence that rounding is not associated with a loss of viability. We therefore concluded that rounding was indicative of trophozoite development rather than mortality.

3.3. Development of rounded trophozoites is triggered by glycoproteins in non-conditioned and HCT-8 conditioned RPMI-FBS

To determine whether glycoproteins in non-conditioned and HCT-8 conditioned RPMI-FBS trigger trophozoite development, we pretreated these media with proteinase K and periodate to digest proteins and oxidize glycans, respectively (Fig. 4). After 6 h in conditioned RPMI-FBS that had been pretreated with proteinase K, $39.0 \pm 2.1\%$ and $42.1 \pm 2.7\%$ of *C. parvum* and *C. hominis*, respectively, were rounded, compared with $99.1 \pm 4.8\%$ and $92.7 \pm 5.6\%$, respectively, in conditioned RPMI-FBS without pretreatment. Similarly, $43.2 \pm 3.8\%$ and $38.0 \pm 3.4\%$ of *C. parvum* and *C. hominis*, respectively, were rounded after 6 h in proteinase K pretreated non-conditioned RPMI-FBS, compared with $70.8 \pm 3.7\%$ and $78.3 \pm 5.3\%$, respectively, without pretreatment. The addition of heat-inactivated proteinase K to non-conditioned and conditioned RPMI-FBS had no effect on trophozoite development (data not shown). Periodate oxidation of glycans had a more dramatic effect on trophozoite development: after 6 h, $3.2 \pm 1.0\%$ and $8.9 \pm 1.7\%$ of *C. parvum* and *C. hominis*, respectively, were rounded in periodate pretreated conditioned RPMI-FBS. Similarly, $1.9 \pm 0.7\%$ and $6.4 \pm 2.7\%$, of *C. parvum* and *C. hominis*, respectively, were rounded in non-conditioned RPMI-FBS pretreated with periodate. The addition of glycerol to non-conditioned and conditioned RPMI-FBS without periodate pretreatment had no effect on trophozoite development (data not shown). These data are evidence that the trigger of trophozoite development in RPMI-FBS and conditioned RPMI-FBS is a glycoprotein.

3.4. Gal-GalNAc triggers trophozoite development and nuclear division in *C. parvum* and *C. hominis*

We next examined the effect of Gal-GalNAc on trophozoite development in *C. parvum* and *C. hominis*. Sporozoites were incubated for 1 and 7 h at 37°C in PBS, non-conditioned RPMI-FBS and conditioned RPMI-FBS, with and without $10 \mu\text{M}$ Gal-GalNAc (Fig. 5). A similar proportion of *C. parvum* zoites became rounded after 1 h in conditioned RPMI-FBS with ($19.1 \pm 2.4\%$) and without ($19.1 \pm 2.3\%$) Gal-GalNAc. For all other 1 h treatments, the inclusion of Gal-GalNAc caused a greater proportion of sporozoites to become rounded ($P < 0.05$), but the proportion was generally less than 5%. The proportion of rounded *C. parvum* and *C. hominis* zoites approached 100% in non-conditioned and conditioned RPMI-FBS after 7 h, whether or not Gal-GalNAc was included. However, a greater proportion of *C. parvum* and *C. hominis* were rounded after 7 h in PBS with Gal-GalNAc ($81.9 \pm 5.7\%$ and

61.9 ± 4.4%, respectively) than without Gal-GalNAc (7.4 ± 1.1% and 4.4 ± 1.1%, respectively).

DAPI staining showed some zoites to have two or more nuclei after 7 h treatments, indicating that nuclear division had taken place (Fig. 6). Most multinucleate zoites were rounded (Fig. 6B), but banana-shaped zoites also were observed (Fig. 6C). Similar proportions of *C. parvum* zoites were multinucleate when Gal-GalNAc was included in PBS (39.2 ± 4.0%), non-conditioned RPMI-FBS (41.8 ± 3.8%), and conditioned RPMI-FBS (36.2 ± 4.8%). When Gal-GalNAc was excluded, the proportion of multinucleate *C. parvum* zoites was greatest in conditioned RPMI-FBS (12.0 ± 2.4%), followed by non-conditioned RPMI-FBS (5.0 ± 1.9%), and PBS (0.39 ± 0.39%) ($P < 0.05$). The proportion of multinucleate *C. parvum* zoites was significantly greater in all treatments that included Gal-GalNAc ($P < 0.05$).

Compared with *C. parvum*, the proportions of multinucleate *C. hominis* zoites were smaller for all treatments ($P < 0.05$). Multinucleate zoites were not detected in PBS without 10 µM Gal-GalNAc, and the proportion of multinucleate zoites was only 1.2 ± 0.5% when 10 µM Gal-GalNAc was included. The proportions of multinucleate *C. hominis* zoites in non-conditioned and conditioned RPMI-FBS, with and without Gal-GalNAc, were similar to that in PBS with Gal-GalNAc ($P > 0.05$).

To determine whether trophozoite development and nuclear division are dependent on the Gal-GalNAc concentration, we treated *C. parvum* and *C. hominis* with 0 µM, 10 µM, 100 µM and 1 mM Gal-GalNAc in PBS (Fig. 7). Increasing the Gal-GalNAc concentration caused a greater proportion of *C. parvum* and *C. hominis* zoites to become rounded ($P < 0.05$). The proportion of *C. hominis* zoites that were multinucleate also increased from 0.9 ± 0.4% at 10 µM Gal-GalNAc to 12.7 ± 3.1% at 1 mM Gal-GalNAc ($P < 0.05$). The proportion of multinucleate *C. parvum* zoites did not vary with Gal-GalNAc concentration.

4. Discussion

Apicomplexans alternate between invasion and replication during development, and efficient switching between these modes contributes to rapid parasite amplification. The switch to replication must be precisely timed because it involves major physiological changes that could compromise invasion if initiated prematurely. Here we have shown that the switch from invasive sporozoite to replicative trophozoite in *Cryptosporidium* is triggered by glycoproteins in FBS and the secretome of HCT-8 cells and by Gal-GalNAc.

FBS triggered trophozoite development in *C. parvum* and *C. hominis* in a concentration-dependent manner, with a transformation rate of approximately 80% in 6 h in 10% FBS. This is more rapid than previously reported in 1% FBS, where trophozoites of *C. parvum* and *C. hominis* were not observed before 24 h (Hijjawi et al., 2004, 2010; Yang et al., 2015). FBS similarly caused trophozoite development in the related apicomplexan *Plasmodium berghei* (Kaiser et al., 2003), a species that also develops intracellular trophozoites in vivo. However, the transformation rate of *P. berghei* trophozoites in 10% FBS (13% in 24 h) was considerably lower than that in the present study.

A report that extracellular *Cryptosporidium* trophozoites can develop in the supernatant from cultured HCT-8 cells (Rosales et al., 2005) prompted us to examine the effect of the HCT-8 cell secretome on trophozoite development. The conditioning step to obtain the secretome required FBS for HCT-8 cell growth, so we were unable to determine whether the secretome can trigger trophozoite development independently of FBS. However, by comparing the effects of non-conditioned and conditioned RPMI-FBS we found that the HCT-8 cell secretome significantly enhances trophozoite development in *C. parvum* but has little effect on *C. hominis*. More rapid trophozoite development in *C. parvum* should result in more rapid replication and a more proliferative infection. Consistent with this, a study by Hashim et al. (2004) showed that *C. parvum* is more proliferative than *C. hominis* in HCT-8 cells, infecting approximately one-third more cells. If the rate of trophozoite development is an important factor in determining the success of an infection, the specificity of the trophozoite trigger could contribute to the host specificity of *Cryptosporidium* spp.

The reduction in trophozoite development following pretreatment of non-conditioned and conditioned RPMI-FBS with proteinase K and periodate was evidence of a glycoprotein trigger. In particular, trophozoite development was almost completely prevented by periodate pretreatment, demonstrating that glycans are critical for trigger activity. We therefore asked whether the glycan Gal-GalNAc that is targeted by *C. parvum* sporozoites during attachment to, and invasion of, host cells (Chen and Larusso, 2000; Bhat et al., 2007) also triggers trophozoite development. We found that Gal-GalNAc triggered not only trophozoite development but also nuclear division in *C. parvum*, indicating that the parasite had transitioned to a replicative mode. While non-conditioned and conditioned RPMI-FBS also triggered replication, which was not detected in our earlier experiments because DAPI staining was not included, these media were less effective triggers than Gal-GalNAc. A possible explanation for this finding is that the Gal-GalNAc epitopes in FBS and the HCT-8 cell secretome were insufficient in number and/or density to meet the threshold to trigger replication. Gal-GalNAc, which is generally covered by other glycan molecules in mucin glycoproteins, is uncovered and reactive in carcinoma cells such as HCT-8 (Springer, 1984), which could explain the greater replication observed when RPMI-FBS was conditioned by HCT-8 cells. Glycoproteins with a sufficient number and density of Gal-GalNAc epitopes to trigger replication may be more abundant in the host cell membrane than the secretome, which would increase the likelihood of differentiation coinciding with attachment and invasion.

Cryptosporidium hominis was less responsive than *C. parvum* to the Gal-GalNAc replication trigger, with only approximately 1% of zoites showing evidence of nuclear division after 7 h in 10 μ M Gal-GalNAc. This is consistent with previous work showing that sporozoite pretreatment with 10 μ M Gal-GalNAc inhibits infection of HCT-8 cells by *C. parvum* but not *C. hominis* (Hashim et al., 2006), and suggests that *C. hominis* may not use Gal-GalNAc to infect HCT-8 cells (Hashim et al., 2006) or to trigger differentiation. Alternatively, *C. hominis* may have a different threshold for Gal-GalNAc mediated attachment and differentiation than *C. parvum*. This is supported by the finding that trophozoite development and replication in *C. hominis* increased with the increasing Gal-GalNAc concentration. In summary, we have identified triggers of the developmental transformation

from an invasive to replicative form in *C. parvum* and *C. hominis*. The ability to induce differentiation outside the host cell opens new opportunities to study regulatory pathways driving the complex developmental changes in the life cycle of *Cryptosporidium* parasites.

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Highlights

- Axenic culture was used to identify triggers of *Cryptosporidium* trophozoite development
- Glycoproteins in FBS trigger trophozoite development in *Cryptosporidium parvum* and *Cryptosporidium hominis*
- Glycoproteins in HCT-8 secretome enhances trophozoite development in *C. parvum*
- Free Gal-GalNAc triggers trophozoite development and nuclear division

Fig. 1.

Sporozoites of *Cryptosporidium parvum* and *Cryptosporidium hominis* become rounded following treatment in RPMI supplemented with 10% FBS (RPMI-FBS), and the rate of *C. parvum* rounding increases when RPMI-FBS is conditioned by HCT-8 cells. (A) *Cryptosporidium parvum* and *C. hominis* sporozoites were treated with RPMI-FBS and HCT-8 conditioned RPMI-FBS (conditioned at 37°C and 5% CO₂ for 3 h.) (B) *Cryptosporidium parvum* zoites under differential interference contrast (DIC) and fluorescence illumination following 3 h in non-conditioned RPMI-FBS and HCT-8 conditioned RPMI-FBS. Note the banana-shaped zoites in the non-conditioned RPMI-FBS and the rounded zoites following treatment with HCT-8 conditioned RPMI-FBS. DAPI-stained nuclei are indicated by arrowheads. Scale bar = 5 μm. (C) Mean length, width and length-to-width ratio of banana-shaped and rounded zoites.

Fig. 2.

FBS causes rounding of sporozoites in a concentration-dependent manner. Sporozoites of *Cryptosporidium parvum* and *Cryptosporidium hominis* were treated at 37°C and 5% CO₂ with RPMI supplemented with 0, 5, 10 or 20% FBS.

Fig. 3.

Rounding is not associated with a loss of viability. The viability of *Cryptosporidium parvum* and *Cryptosporidium hominis* zoites was determined using fluorescein diacetate-propidium iodide staining following treatment with non-conditioned or HCT-8 conditioned RPMI-FBS at 37°C and 5% CO₂ for periods ranging from 3 to 12 h.

Fig. 4.

A glycoprotein in non-conditioned and HCT-8 conditioned RPMI-FBS causes rounding. Sporozoites of *Cryptosporidium parvum* and *Cryptosporidium hominis* were treated with non-conditioned and HCT-8 conditioned RPMI-FBS without pretreatment, following pretreatment with proteinase K, or following pretreatment with sodium metaperiodate.

Fig. 5.

Treatment with 10 μ M Gal-GalNAc causes rounding of *Cryptosporidium parvum* and *Cryptosporidium hominis* sporozoites. Sporozoites of *C. parvum* and *C. hominis* were treated for 1 or 7 h with PBS, non-conditioned RPMI-FBS or HCT-8 conditioned RPMI-FBS, with and without 10 μ M Gal-GalNAc.

Fig. 6.

Treatment with 10 μ M Gal-GalNAc causes nuclear division. (A) Sporozoites of *Cryptosporidium parvum* and *Cryptosporidium hominis* were treated for 7 h with PBS, non-conditioned RPMI-FBS or HCT-8 conditioned RPMI-FBS, with and without 10 μ M Gal-GalNAc. (B) Rounded *C. parvum* zoite with two nuclei (arrowheads). (C) Banana-shaped *C. parvum* zoites with two nuclei (arrowheads). Scale bar = 5 μ m.

Fig. 7.

The proportions of rounded *Cryptosporidium parvum* and *Cryptosporidium hominis* zoites and the proportion of multinucleate *C. hominis* zoites increase with increasing Gal-GalNAc concentration. Sporozoites of *C. parvum* and *C. hominis* were treated for 7 h with 0 μM, 10 μM, 100 μM and 1 mM Gal-GalNAc in PBS.