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Cryptosporidium parvum oocytic antigen induces dendritic cell maturation that suppresses Th2 cytokines when co-cultured with CD4$^+$ cells

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Abstract

Cryptosporidium parvum is an opportunistic intracellular parasite that causes disease in animal populations such as calves and goats. It is also a significant zoonotic disease globally, causing mild to severe human diarrhea. In immunocompromised animals, calves and lambs, and immunocompromised humans such as AIDS patients, an infection can be life-threatening as no effective treatments are currently available to control infection. The effects of *Cryptosporidium parvum* antigen (CPA) on dendritic cells (DCs) were investigated. This study examined cytokine secretion and cell surface marker expression on DCs exposed to CPA. Cytokine production in CD4$^+$ cells co-cultured with CPA primed DCs in the presence of anti-CD3 was also measured. CPA induced a significant increase in the production of interleukin (IL)-12p40, IL-10, IL-6, and TNF-α by DCs and enhanced the expression of the cell surface markers TLR4, CD80, CD86, and MHC11. CPA primed DC co-cultured in the presence of anti-CD3 with CD4$^+$ T-cells inhibited the secretion of Th2-associated cytokines, notably IL-5 and IL-13, with no effects on the secretions of interferon (IFN)-γ, IL-2, IL-17, and IL-10. These findings support studies in the literature that CPA can induce the full maturation of DCs that subsequently initiate Th1 immune responses critical to the resolution of *C. parvum* infection.

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Introduction

Cryptosporidiosis was identified at first as a veterinary disease of clinical concern in domestic animals such as cattle, buffaloes, and goats (1-3). *C. parvum* infection in calves is a primary veterinary concern, especially in temperate regions, because it causes watery diarrhea, in appetence, tiredness, dehydration, and death. Long-term growth of *Cryptosporidium*-affected calves can be costly for farmers due to lost income from lower carcass weights, treatment expenditures, and increased feed requirements to get calves to market weight (4). There remains a paucity of data for other farmed animals, despite *Cryptosporidium* infection causing significant clinical disease. *C. parvum* is also a crucial zoonotic parasite that can infect humans through transmission from infected people or animals or indirectly by ingesting contaminated food or water (5). While this intracellular coccidian parasite has a worldwide distribution, it is especially prevalent in humans and livestock of economic importance in Egypt (6,7). The highest prevalence rates of infection are along the Nile River in rural communities that reside near infected animals (8). A current review by Helmy (9) reported that the prevalence rate in Egypt in animals varied between 2% and 69%, demonstrating that animals are an essential vector for this parasite, the prevalence of *Cryptosporidium* spp. in the calves being significantly higher than in adult cattle. The prevalence rates in human populations varied from 3% to 50%, with reports of up to 91% in immunocompromised cohorts such as children with diarrheal disease (9,10).

Transmission of *C. parvum* occurs by ingestion of parasitic oocysts consumed in drinking water or food contaminated with infected fecal matter (11,12). Oocysts enter epithelial cells that line the gastrointestinal tract causing symptoms such as low-grade fever, nausea, diarrhea, and weight loss (13). Disease symptoms are self-limiting in immune-competent animals and humans. However, in immune-compromised individuals (14,15), the infection can be life-threatening, exacerbated by a deficiency in effective therapies (15,16). To date, there are no effective vaccines due to the incomplete understanding of the host immune response to the parasite infection (17,18). A better understanding of the host's innate and adaptive immune responses to infection might contribute to developing a *C. parvum* vaccine.

Similar to other protozoan infections, immune responses associated with *C. parvum* infection involve a complex interplay between the innate and adaptive immune responses. Cytokines such as IL-12 and IFN-ɤ that are associated with *C. parvum* infection are essential mediators of Th1 responses (19,20). Antigen-presenting cells exposed to *C. parvum* or its antigenic products secrete Th1-promoting cytokines like IL-12p35 or IL-12p40, which can differentiate naïve $CD4^+$ T-cells into a Th1 phenotype (20). Studies also show that IFN-ɤ deficient mice are susceptible to *C. parvum* infection (21,22), indicating that IL-12 and IFN-ɤ are central to developing protective immunity during *C. parvum* infection. Moreover, the regulatory cytokine IL-10 is induced during infection as part of regulatory networks to dampen intestinal inflammation (23). Host cell-mediated immune responses are thought to be critical during infection, specifically $CD4^+$ T-cells during recovery from cryptosporidial infections (24,25).

DCs, as antigen-presenting cells, play a significant role in activating innate and adaptive immune responses in infected hosts (26), processing and presenting the parasite antigens in local lymph nodes to T- and B-cells (27). Upon antigen stimulation, immature DCs begin to mature and migrate into the lymph nodes and other immune organs, causing the upregulating of expression of the major histocompatibility complex (MHC)-I, MHC-II, CD86 with other costimulatory factors, as well as the expression of pro-inflammatory cytokines that are important for the induction of adaptive immune responses (28). Mice are a good model for studying the effect of cryptosporidium infection in DCs (20,29). Little is known about the role of DCs in cryptosporidiosis; they are involved in the degradation and transport of antigens to lymph nodes (27) and are known to release chemokines in response to *C. parvum* infection (30). Wanyiri and Ward (31) examined dendritic cell activation by recombinant *C. parvum* antigens. Also, Bedi and Mead (20) study have shown that *C. parvum* sporozoite activated dendritic cells and produced Th1 cytokines. Xu *et al.* (32) indicated that DCs pulsed with live sporozoites in vitro and co-cultured with CD4+ and CD8+ T cells produced higher IFN- levels and showed induction of Th1 immune response.

While T cell-mediated immunity appears essential in controlling *Cryptosporidium* infection, the mechanisms that elicit these immune responses are unclear. Given their importance in dealing with C. *parvum* infections, we investigated the effects of *C. parvum* oocysts antigen on bone marrow-derived DCs. These studies are essential because there are limited studies about the effects *of C. parvum* oocysts antigen. This study demonstrated that DCs exposed to *C. parvum* oocysts induced a pro-inflammatory DC phenotype that suppressed Th2 cytokines from T-cells while the secretion of cytokines associated with Th1, Th17, and regulatory phenotypes remained unchanged. This interaction led to DC activation as determined by the overexpression of several costimulatory molecules and cytokines. The proposed studies will provide the basis for understanding the mechanisms for the induction of DCs and T cell-mediated anti-*Cryptosporidium* immunity, which are necessary for future vaccine design and other effective methods for treatment.

Materials and methods

Animals and ethical standards

Balb/c mice (female) aged 6-8 weeks were purchased from Charles River Ltd (Kent, UK) and kept under specific pathogen-free conditions at Dublin City University (DCU). All mice were housed according to the Health Products Regulatory Authority (HPRA) guidelines with strict adherence to standard operating procedures approved by the Institutional Animal Welfare Body. Ethical permission for the use of animals was approved by the Health Products Regulatory Authority and Dublin City University ethics committee (license number DCUREC/2010/033). All procedures involving animals were only performed by licensed personnel licensed by the HPRA.

Animals were culled under the ARRIVE Guidelines relevant to *ex vivo* models (33). For each experiment, the absolute minimum number of animals was culled. No procedures or processes were performed on the animal's pre-mortem. As all investigations were *ex vivo* in nature, the average number of cell types yielded per mouse was calculated and used to determine the number of mice needed; where appropriate multiple cell types were harvested to ensure maximum yield from each animal and minimize animal numbers. Animals were chosen from the same group and age bracket - they are housed based on these criteria. Animals were included once they were of the same group and healthy in appearance.

Cryptosporidium parvum oocysts antigen preparation

The *C. parvum* antigen (CPA) was prepared from *C. parvum* oocysts purified from naturally infected calves (34). In brief, oocysts were purified from fecal samples by

sucrose and percoll centrifugation, treated in 0.5% sodium hypochlorite solution at 4°C for 10 min, washed 4 times in sterile water, and re-suspended in PBS at 2 × 10^8 oocysts/mL. Purified oocytes were homogenized in sterile PBS (pH 7.4) and centrifuged for 30 min at 876 g at 13,000 rpm. The supernatant antigen was collected, aliquoted, and stored at -20 until use. The protein concentration was measured using the lowery method (35). Endotoxin levels were tested for all antigens and were less than the lower detection limit in this assay (<0.01 EU/ml).

Reagents and materials

Lipopolysaccharide (LPS) from *E. coli* (serotype R515) was purchased from Enzo Life Sciences (Exeter, UK). All antibodies used in this investigation were obtained from eBiosciences (Hatfield, UK; CD86 (FITC) monoclonal antibody (24F), CD80 (PE) monoclonal antibody (3H5), MHCII (FITC) monoclonal antibody (MS/114.15.2) and TLR4 (PE) monoclonal antibody (UT41) or the relative isotype control. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was obtained from Sigma Aldrich. Cell culture materials were purchased from Biosciences (Dun Laoghaire, Ireland).

Isolation and culture of bone marrow-derived dendritic cells

Bone marrow-derived DCs (BMDCs) obtained from balb/c mice (36) were isolated aseptically in a Class II Laminar cabinet (ThermoElectron Corporation, USA). Bone marrow cells were extracted from the tibia and femurs of each mouse by flushing the bone cavity with sterile RPMI using a sterile 25.7 g needle and syringe. The cells were pelleted by centrifuging for 5 min at 500 g and re-suspended in 10 mL of culture medium (RPMI (Gibco, UK) supplemented with 20% heat-inactivated fetal bovine serum (FBS), 100 µg/ml penicillin/streptomycin (Invitrogen, UK), L-glutamine, and 50 ng/mL granulocyte monocyte-colony stimulating factor (GM-CSF) (Sigma Aldrich, Ireland)). Cells were transferred to a petri dish and cultured a 37°C in a CO_2 incubator. On days 3 and 6, 6 mL of media was gently removed from the petri dish, and the media was replenished with 10 mL of pre-warmed culture medium. On day 10, adherent cells were dislodged from the surface using a cell scraper (Sarstedt, Ireland) and centrifuged at 500 g for 5 mins prior to resuspension in fresh media. Cell counting was performed using the trypan blue exclusion method. Harvested BMDCs were analyzed by flow cytometry, and only cell preparations with a population identified as >95% CD11C (Biolegend, No. 117317) positive were used for each experiment.

3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl) -2-(4-sulfophenyl) -2H-tetrazolium (MTS) Assay

CellTiter 96® Aqueous One Solution (Pierce, UK) test was used to investigate the cytotoxic effects of CPA on BMDCs *in vitro* (37). BMDCs were plated in a 96-well plate (Nunc™, Ireland) with 100 µL cell suspension per well at a concentration of $1x10^6$ cells/mL. CPA was added at increasing concentrations (10 - 10,000 ng/mL) or cells were plated with media, LPS (100 ng/mL) or DMSO (10% v/v). Cells were incubated overnight at 37°C in a CO_2 incubator, and then 20 µL of the CellTiter 96® Aqueous One solution was added to each well. After 4 hrs, the color change in the media was measured using a TECAN 96 well plate reader at 490 nm (Tecan, Männedorf, Switzerland). The cell viability of each sample was calculated by setting the absorbance value for the cells treated with media alone as a reference point, and then the percentage change in absorbance for each sample was calculated as previously described (37).

Enzyme linked immunosorbent assay

BMDCs (1 x 10^6 cells/mL) were treated with CPA (10 and 100 ng/mL), LPS (100 ng/mL) or media and incubated for 18 hrs at 37°C in a CO_2 incubator. Supernatants were removed, and cytokine release (IL-12p40, IL-6, and IL-10) was measured using commercial ELISA kits following the manufacturer's instructions (Invitrogen, UK). Each sample and standard were assayed in triplicate.

Flow cytometry

BMDCs (1 x 10^6 cells/mL) were treated with CPA (10 and 100 ng/mL), LPS (100 ng/mL) or media and incubated at 37°C in a CO_2 incubator for 24 hrs. Cells were removed from the tissue culture plates and placed into a 96-well round bottom plate at 400,000 cells/well. An equal amount of FBS was subsequently added for 15 min at room temperature (RT) to block non-specific binding. Cells were then washed three times with FACS buffer (PBS supplemented with 2% FBS (v/v) and 1mM EDTA (Sigma-Aldrich)) and incubated with appropriate fluorochrome-conjugated antibodies (BD Biosciences, UK) for 30 min at 4ºC while protected from light. Cells were washed three times to remove any unbound antibodies and analyzed using a FACSAria I (BD Biosciences, UK). All the flow cytometry data were analyzed using FlowJo software (Treestar, UK).

T-cell co-culture

Spleens from balb/c mice were harvested, and splenocytes were obtained by passaging the spleen through a 40 µm filter (Sarstedt, Nümbrecht, Germany) using the plunger from a sterile 1 mL syringe (Sarstedt, Nümbrecht, Germany). $CD4^+$ T-cells were isolated from the splenocytes using a negative selection $CD4^+$ isolation kit (Stemcell, Vancouver, Canada) and were only used if the purity was determined to be > 95% positive for $CD4^+$ cells by flow cytometry. BMDCs were stimulated with CPA (10 ng/ml) for 24 hrs, washed in PBS three times, and co-cultured with $CD4^+$ T-cells at a ratio of 1:10 in a culture medium on 24

healthy plates (Sarstedt, Nümbrecht, Germany) that had been pre-coated overnight with anti-CD3 (1 µg/mL) (R and D systems, Minneapolis, Minnesota, USA). Supernatants were harvested after 72 hrs and analyzed for cytokine release (IL-5, IL-13, IFN-γ, IL-2, IL-17, and IL-10) using commercial ELISA kits following the manufacturer's instructions (Invitrogen, UK). Each sample and standard were assayed in triplicate.

Statistical analysis

All data were analyzed for normality prior to statistical testing by Prism® 6.0 (GraphPad Software Inc, La Jolla, CA, USA) software. Data were analyzed using one-way ANOVA using Tukey's multiple comparison test, where multiple group comparisons were made. For comparisons between two groups, the student's *t*-test was used. Data are expressed as mean ± standard deviation (SD).

Results

Lower doses of *C. parvum* antigen do not exhibit cytotoxic effects on BMDCs

Before examining the immune properties of CPA on BMDCs, the cytotoxic effect of the antigen on these cells was assessed using an MTS Assay. At lower concentrations (10, 100, and 500 ng/mL), CPA had no significant impact on cell viability. However, at higher doses, CPA (1000 ng/mL ($P \leq 0.05$) and 10,000 ng/mL ($P \leq 0.01$)) was cytotoxic, with the highest dose exhibiting cytotoxic effects similar to DMSO (10%), the positive control (Figure 1). Since 1000 ng/mL and 10,000 ng/mL were displayed cytotoxic effects on BMDCs. These antigen concentrations were excluded from subsequent assays.

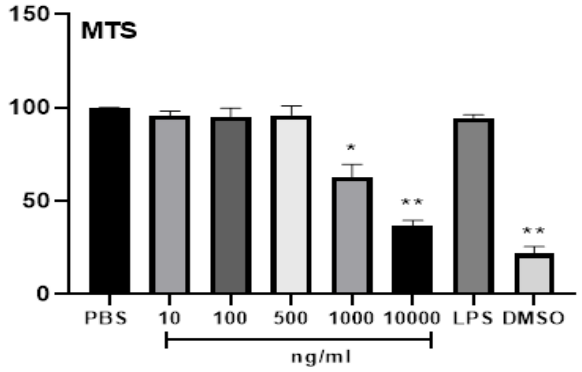

Figure 1: MTS assay assessed the cytotoxic effects exerted by increasing concentrations of CPA on BMDCs (10 to 10,000 ng/ml). The graph represents cell viability, expressed as absorbance ± SD of three independent experiments. P-values were calculated using one-way ANOVA. *, $P \leq 0.05$; **, $P \leq 0.01$ compared to PBS control group.

***Cryptosporidium parvum* antigen activates BMDCs to produce a panel of pro-inflammatory cytokines**

To understand the immuno-modulatory properties of CPA, BMDCs were stimulated with three different concentrations of antigen (10, 100, and 500 ng/mL), and cytokine release was measured 18 hrs later. The pro-inflammatory cytokines IL-12p40, TNFa, and IL-6 associated with protozoan infection and IL-10, an anti-inflammatory cytokine that regulates inflammatory processes during infection. CPA induced significant secretion of IL-12p40, TNFa, and IL-10 from BMDCs in a dose-dependent manner with no significant secretion of IL-6 (Figure 2).

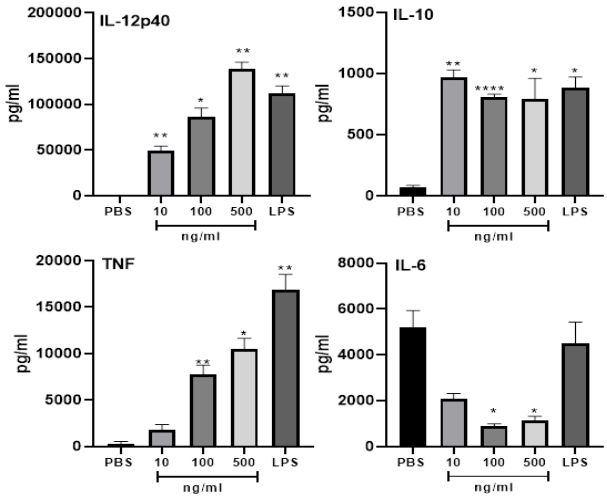

Figure 2: BMDCs were stimulated with increasing concentrations of CPA (10 - 500 ng/mL) and analyzed for the secretion of IL-12p40, IL-10, TNFα, and IL-6 by commercial ELISA after 18 hours. Results are expressed as mean ±SD of three independent experiments. P-values were calculated using one-way ANOVA. *, $P \leq 0.05$; **, $P \leq 0.01$: ***, $P \leq 0.001$; ****, $P \leq 0.0001$ compared to PBS control group.

BMDCs treated with CPA express costimulatory markers.

Since CPA stimulated BMDCs secreted a panel of cytokines, we examined their effect on BMDC maturation. BMDCs were stimulated with different concentrations of CPA (10 - 500 ng/mL), and after 18 hours, the expression of the cell surface markers CD80, CD86, MHC-11, and TLR4 were measured. Significant expression of all cell surface markers was observed for all antigen doses tested except for CD80, where only the lowest concentration induced CD80 expression (Figure 3). It was unexpected that only the lower dose of CPA induces the expression of CD80. Although there is a slight increase at higher levels, it is not statistically significant. This could be explained by

the fact that these bioassays are affected by numerous factors. For example, the oocyte mixture is a heterogeneous mixture of antigens that at higher concentrations activate other pathways where CD80 is not induced.

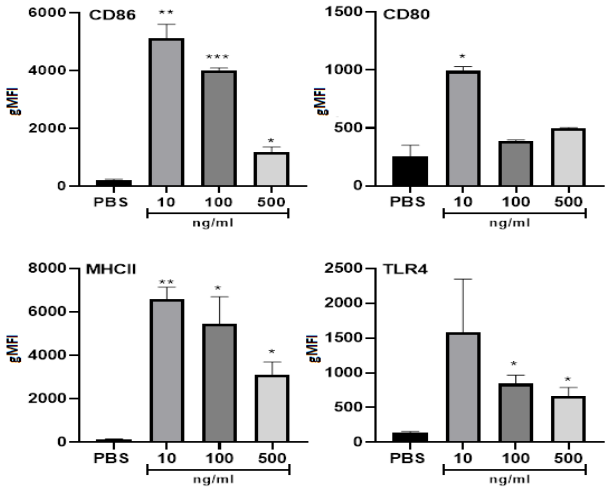

Figure 3: BMDCs were stimulated at different concentrations of CPA (10 - 500 ng/mL) for 18 hours and subsequently assessed for the expression of CD86, CD80, and MHCII by flow cytometry following staining for 30 min with specific antibodies or an isotype-matched control. Results were analyzed using FlowJo software (Treestar, USA) and are expressed as the geometric MFI ±SD of three independent experiments. P-values were calculated using a two-tailed student's t-test. n.s, non-significant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ compared to PBS control.

Dendritic cells stimulated with C. *parvum* antigen suppress Th2 cytokines.

Since CPA induced maturation of BMDCs, we then examined the priming of naïve $CD4^+$ T-cells by CPA-stimulated BMDCs. BMDCs stimulated with CPA were co-cultured with $CD4^+$ cells in the presence of plate-bound anti-CD3. $CD4^+$ cells secreted significantly less IL-13 and IL-5 (Figure 4) compared to the control. No significant differences in IFN-γ , IL-2, IL-17, and IL-10 secretion were observed (Figure 4).

Discussion

Cryptosporidiosis is a primary veterinary concern due to its global distribution affecting various animal species (2). While there are over 26 *Cryptosporidium* species in Egypt, the overall estimated prevalence of *Cryptosporidium* infection in ruminants was 32.2%, with *C. parvum* accounting for 65.7% of the species that infect cattle and buffalos, livestock of economic importance in Egypt (9). *C. hominis* and *C. parvum* are responsible for more than 90% of human cryptosporidiosis cases, and there is a clear zoonotic effect between infected humans and animals. The present study demonstrated that CPA induced maturation of pro-inflammatory-like DCs that suppressed Th2 cytokines when co-cultured with $CD4^+$ cells. This DC phenotype involving the secretion of IL-12 and TNFα is typically associated with protozoan infection (38,39).

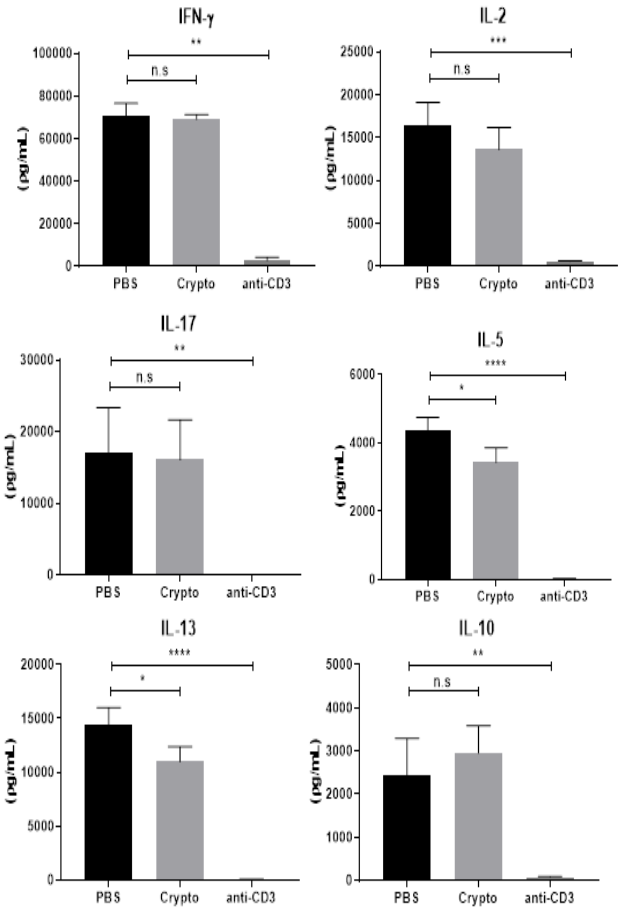

Figure 4: BMDCs pre-treated with CPA (10 ng/mL) or PBS were co-cultured with $CD4^+$ T-cells at a ratio of 1:10 on plates that had been pre-coated with anti-CD3 (1 µg/well) overnight. $CD4^+$ T-cells alone with anti-CD3 have used a negative control. After 72 hrs, supernatants were analyzed for the secretion of IFN-γ, IL-2, IL-17, IL-5, IL-13, and IL-10 by commercial ELISA kits. Results are expressed as mean ±SD of three independent experiments. P-values were calculated using tukey one-way ANOVA. n.s, non-significant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ compared to PBS control group.

In the current study, treating BMDCs with CPA induced the secretion of the pro-inflammatory cytokines TNFα, IL-6, and IL-12p40, and the anti-inflammatory cytokine IL-10. The critical role of IL-12p40 was confirmed by Ehigiator *et*

al. (40), who showed that IL-12p40$^{-/-}$ mice were more susceptible to infection with *C. parvum* than control mice. IL-12 plays an essential role during *C. parvum* infection in the differentiation of Th1 cells and the subsequent production of IFN-ɤ, which is essential in controlling *C. parvum* replication (41-43). Mice with a targeted mutation in the IL-12p40 gene were susceptible to *C. parvum* (44). IL-12p40, together with a p19 subunit, makes up the IL-23 cytokine. This cytokine drives the development of T helper cells that secrete IL-17 (Th17), and these cells are also found to have a role in the elimination of cryptosporidium infection (45).

In contrast to the effects of IL-12p40, IL-6 has been shown to directly antagonize the induction of Th1 responses by upregulating the expression of signaling factors that interfere with IFN-γ signaling and induce the differentiation of T-cells towards a Th2 phenotype (46). Despite the known import of Th1 responses and cytokines in clearing *C. parvum* infection, IL-6 has been reported to also play an essential role in the clearance of Cryptosporidium (47), suggesting that a mixed Th1/2 response may be required to fully clear infection. The present result shows that DC exposure to CPA increases the production of IL6, which coincided with that observed by Bedi and Mead (20) and Xu *et al.* (32). They illustrated an increase in the expression of IL-6 by mouse DCs treated with C. parvum sporozoite antigen which was dependent on TLR signaling pathways. TNF-α can inhibit *C. parvum* development in enterocyte cell lines and is thought to limit parasite replication within the host (48,49). The production of IL-10 may be part of a negative feedback mechanism to limit tissue damage from these pro-inflammatory responses induced by the parasite (50).

Lower concentrations of *C. parvum* antigens activated DCs, keeping with previous studies that also demonstrated that *C. parvum* antigens activate mouse and human DCs at lower antigen concentrations (20). This study also supports findings by Bedi and Mead (20), who reported significant increases in the expression of IL-12p70, IL-2, and IL-1β from mouse DCs in response to *C. parvum* sporozoite antigen. Similarly, they showed that BMDCs stimulated with *C. parvum* sporozoite lysate and live antigen preparations had little effect on IL-6 production. While, Perez-Cordon *et al.* (51) demonstrated that *C. parvum* antigens induced IL-12 and TNF-α from murine BMDCs but also reported an increase in IL-6, which contradicts our findings. This difference may be due to the type of parasite isolate used in their study or the excystation technique used to prepare the oocysts. A recent study has shown that different parasite isolates induce differences in immune response in studies on antigen-presenting cells *in vitro* (52).

CPA induced the full maturation of DCs and enhanced cytokine secretion. Enhanced expression of cell surface markers is associated with DC maturation and is vital for activating CD4$^+$ cells. The enhanced expression of TLR4 is consistent with previous studies, as TLR4 is essential for *C. parvum*-induced NF-kB activation in human biliary epithelial cells (53). It has been shown that the production of Th1 cytokines by *C. parvum*-treated DCs was MyD88 dependent (20). Moreover, the production of IL-12 by *C. parvum*-infected BMDCs from C3H/HeJ mice (which lack a functional TLR4 pathway) was defective, suggesting that TLR4 signaling is vital for the production of IL-12 by *C. parvum*-infected BMDCs (51). Our findings are also supported by Perez-Cordon *et al.* (51). They reported that the oral exposure of mice to *C. parvum* resulted in DC maturation characterized by reduced endocytosis and an augmented expression of MHC molecules, costimulatory molecules, and adhesion molecules. Following *in vitro* infection of mouse BMDCs with *C. parvum* sporozoites, DCs increased their expression of CD40, CD80, and CD86. CD4$^+$ cell activation is critical during *C. parvum* infection as MHC class II deficient mice (which lack functional CD4$^+$ T cells) infected with *C. parvum* develop unresolved chronic infection (54).

DCs are essential players during early host immune response, and their depletion results in significantly increased susceptibility to infection with *C. parvum* (55). Adoptive transfer of unstimulated or *C. parvum* antigen-stimulated DCs into CD11c$^+$ depleted CD11c-DTR-Tg mice resulted in an early decrease in parasite load at 4 days post-infection. However, this response was transient since parasite load increased in mice engrafted with either unstimulated DCs or DCs stimulated with solubilized antigen 6 days post-infection. In contrast, in mice engrafted with DCs stimulated with live sporozoites, parasite load remained low during the entire period, suggesting the development of a more effective and sustained response (55). The infection with *C. parvum* infection requires CD4$^+$ T cell, major histocompatibility complex (MHCII) (56), and another signaling pathways like CD154 and CD40 that are highly expressed on DCs (57).

The host defense against cryptosporidiosis has been shown to depend upon a combination of innate and adaptive mediated immunity (25). There are interleukins as IL-1, IL-2, IL-15, IL-6, IL-8, IL-5, IFN-γ, IL-4 were induced in *C. parvum* infection (58). This current study showed that BMDCs matured with CPA suppressed the Th2-associated cytokines, IL-5 and IL-13, while simultaneously exhibiting no effect on IFNɤ, IL-2, IL-17, and IL-10. The suppression of Th2 cytokines results in dominant Th1/Th17 responses typically associated with *Cryptosporidium* infection. These results differed from studies by Bedi *et al.* (55), who demonstrated an increase in IFN when DCs stimulated with CPA were adoptively transported into mice. Also, Xu *et al.* (59) illustrated that *C. parvum* infection results in high expression levels of IFN-γ in the peripheral blood. However, the in vivo situation is more complex, suggesting that other factors are critical to inducing IFN-γ that could not be replicated in an *in vitro* assay. IFN-γ is a critical

factor during Cryptosporidiosis infection in human populations, where studies have observed a more severe infection in populations unable to produce IFN-γ . The role of IFN-γ during the early stages of infection appears to be critical in controlling parasite replication (25,60). Xu *et al.* (32) show the ability of activated DCs to release IL-12 and IFN-γ in response to *C. parvum*. Also, IFN-γ knockout mice suffered a more severe infection of *C. parvum* than the control mice (40).

Conclusion

In conclusion, in the present study, we investigated the effect of *C. parvum* antigen on the activation of DCs. The presented results indicate that the interaction between DCs and *C. parvum* antigen results in a fully mature DC phenotype that secretes IL-12 p40, TNF-α, IL-6, and IL-10 and expresses MHCII TLR4, CD80, and CD86. The *C. parvum* antigen contributed to the predominant production of Th1 cytokines and inhibiting Th2 cytokine production. The findings from this study support other studies reported in the literature and highlight the importance of Th1 immunity during host-parasite interactions.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Efstratiou A, Ongerth JE, Karanis P. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks-an update 2011-2016. Water Res. 2017;114:14-22. DOI: 10.1016/j.watres.2017.01.036
2. Pumipuntu N, Piratae S. Cryptosporidiosis: A zoonotic disease concern. Vet World. 2018;11:681-686. DOI: 10.14202/vetworld.2018.681-686
3. Barnes AN, Davaasuren A, Baasandavga U, Lantos PM, Gonchigoo B, Gray GC. Zoonotic enteric parasites in Mongolian people, animals, and the environment: Using one health to address shared pathogens. PLOS Negl Trop Dis. 2021;115:e0009543. DOI: 10.1371/journal.pntd.0009543
4. Luhanda F, Irunde JI, Kuznetsov D. Modeling cryptosporidiosis in humans and cattle: Deterministic and stochastic approaches. Parasit Epidemiol Control. 2023;293. DOI: 10.1016/j.parepi.2023.e00293
5. Delling C, Daugschies A. Literature review: Coinfection in young ruminant livestock—*Cryptosporidium spp.* and its companions. Pathogens. 2022;11:103. DOI: 10.3390/pathogens11010103
6. Thomson S, Hamilton CA, Hope JC, Katzer F, Mabbott NA, Morrison LJ, Innes EA. Bovine cryptosporidiosis: Impact, host-parasite interaction, and control strategies. Vet Res. 2017;48:42. DOI: 10.1186/s13567-017-0447-0
7. Elmonir W, Elaadli H, Amer A, El-Sharkawy H, Bessat M, Mahmoud SF, El-Tras WF. Prevalence of parasitic intestinal infections and their associated risk factors among preschool and school children in Egypt. Plos One. 2021;16:e0258037. DOI: 10.1371/journal.pone.0258037
8. Elshazly AM, Elsheikha HM, Soltan DM, Mohammad KA, Morsy TA. Protozoal pollution of surface water sources in Dakahlia governorate, Egypt. J Egypt Soc Parasitol. 2007;37:51-64. [available at]
9. Helmy YA, El-Adawy H, Abdelwhab EM. A comprehensive review of common bacterial, parasitic and viral zoonoses at the human-animal interface in Egypt. Pathogens. 2017;6:33. DOI: 10.3390/pathogens6030033
10. Abdel Gawad SS, Ismail MM, Imam NA, Eassa AA, Abu-Sarea EY. Detection of *Cryptosporidium spp*. in diarrheic immunocompetent patients in Beni-Suef, Egypt: Insight into epidemiology and diagnosis. Korean J Parasitol. 2018;56:113-119. DOI: 10.3347/kjp.2018.56.2.113
11. Li J, Shi K, Sun F, Li T, Wang R, Zhang S, Jian F, Ning C, Zhang L. Identification of human pathogenic *Enterocytozoon bieneusi*, *Cyclospora cayetanensis*, and *Cryptosporidium parvum* on the surfaces of vegetables and fruits in Henan, China. Int J Food Microbiol. 2019;307:108292. DOI: 10.1016/j.ijfoodmicro.2019.108292
12. Fresán, MA, Pliego AB. The role of cryptosporidiosis in sheep welfare. Open access peer-reviewed chapter -ONLINE FIRST 2021. DOI: 10.5772/intechopen.99876.
13. Carter B, Stiff R, Elwin K, Hutchings H, Mason B, Davies A, Chalmers R. Health sequelae of human cryptosporidiosis—a 12-month prospective follow-up study. Eur J Clin Microbiol Infect Dis. 2019;38:1709-1717. DOI: 10.1007/s10096-019-03603-1
14. Cacciò SM, Chalmers RM. Human cryptosporidiosis in Europe. Clin Microbiol Infect. 2016;22:471-80. DOI: 10.1016/j.cmi.2016.04.021
15. Tomczak E, McDougal AN, White Jr AC. Resolution of cryptosporidiosis in transplant recipients: Review of the literature and presentation of a renal transplant patient treated with nitazoxanide, azithromycin, and rifaximin. Open Forum Infect Dis. 2021;4:9.DOI: 10.1093/ofid/ofab610
16. Khalil IA, Troeger C, Rao PC, Blacker BF, Brown A, Brewer TG, Colombara DV, De Hostos EL, Engmann C, Guerrant RL, Haque R. Morbidity, mortality, and long-term consequences associated with diarrhea from cryptosporidium infection in children younger than 5 years: A meta-analyses study. Lancet Glob Health. 2018;6:e758-e768. DOI: 10.1016/S2214-109X(18)30283-3
17. Chen XM, Keithly JS, Paya CV, LaRusso NF. Cryptosporidiosis new England. J Med. 2002;346:1723-1731. DOI: 10.1056/NEJMra013170
18. Checkley W, White AC Jr, Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, Fayer R, Griffiths JK, Guerrant RL, Hedstrom L, Huston CD, Kotloff KL, Kang G, Mead JR, Miller M, Petri WA Jr, Priest JW, Roos DS, Striepen B, Thompson RC, Ward HD, Van Voorhis WA, Xiao L, Zhu G, Houpt ER. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. Lancet Infect Dis. 2015;15:85-94. DOI: 10.1016/S1473-3099(14)70772-8
19. Tessema TS, Schwamb B, Lochner M, Förster I, Jakobi V, Petry F. Dynamics of gut mucosal and systemic Th1/Th2 cytokine responses in interferon-gamma and interleukin-12p40 knock out mice during primary and challenge *Cryptosporidium parvum*. Infect Immunobiol. 2009;214:454-466. DOI: 10.1016/j.imbio.2008.11.015
20. Bedi B, Mead J. *Cryptosporidium parvum* antigens induce mouse and human dendritic cells to generate Th1-enhancing cytokines. Parasite immunol. 2012;34:473-485. DOI: 10.1111/j.1365-3024.2012.01382.x
21. Ehigiator H, Romagnoli P, Borgelt K, Fernandez M, McNair N, Secor W, Mead J. Mucosal cytokine and antigen-specific responses to *Cryptosporidium parvum* in IL-12p40 KO mice. Parasite immunol. 2005;27:17-28. DOI: 10.1111/j.1365-3024.2005.00736.x

22. Jakobi V, Petry F. Humoral immune response in IL-12 and IFN-γ deficient mice after infection with *Cryptosporidium parvum*. Parasite immunol. 2008;30:151-161. DOI: 10.1111/j.1365-3024.2007.01013.x
23. Laurent F, Lacroix-Lamandé S. Innate immune responses play a key role in controlling infection of the intestinal epithelium by cryptosporidium. Int J Parasitol. 2017;47:711-721. DOI: 10.1016/j.ijpara.2017.08.001
24. McDonald V, Bancroft G. Mechanisms of innate and acquired resistance to *Cryptosporidium parvum* infection in SCID mice. Parasite immunol. 1994;16:315-320. DOI: 10.1111/j.1365-3024.1994.tb00354.x
25. Gomez Morales MA, Mele R, Ludovisi A, Bruschi F, Tosini F, Pozio E. *Cryptosporidium parvum*-specific CD4 Th1 cells from sensitized donors responding to both fractionated and recombinant antigenic proteins. Infect immun. 2004;72:1306-1310. DOI: 10.1128/IAI.72.3.1306-1310.2004
26. Walsh KP, Mills KH. Dendritic cells and other innate determinants of T helper cell polarisation. Trends immunol. 2013;34:521-530. DOI: 10.1016/j.it.2013.07.006
27. Ponnuraj EM, Hayward AR. Intact intestinal mRNAs and intestinal epithelial cell esterase, but not *Cryptosporidium parvum*, reach mesenteric lymph nodes of infected mice. J Immunol. 2001;167:5321-5328. DOI: 10.4049/jimmunol.167.9.5321
28. Li J, Wang X, Wang W, Luo J, Aipire A, Li J, Zhang F. *Pleurotus ferulae* water extract enhances the maturation and function of murine bone marrow-derived dendritic cells through the TLR4 signaling pathway. Vaccine. 2015;33:1923-1933. DOI: 10.1016/j.vaccine.2015.02.063
29. O'Hara SP, Bogert PS, Trussoni CE, Chen X, LaRusso NF. TLR4 promotes *Cryptosporidium parvum* clearance in a mouse model of biliary cryptosporidiosis. J Parasitol. 2011;97:813-821. DOI: 10.1645/GE-2703.1
30. Auray G, Lacroix-Lamande S, Mancassola R, Dimier-Poisson I, Laurent F. Involvement of intestinal epithelial cells in dendritic cell recruitment during *C. parvum* infection. Microbes Infect. 2007;9:574- 582. DOI: 10.1016/j.micinf.2007.01.026
31. Wanyiri J, Ward H. Molecular basis of Cryptosporidium-host cell interactions: Recent advances and future prospects. Future Microbiol. 2006;1:201-208. DOI: 10.2217/17460913.1.2.201
32. Xu L, Kwak M, Zhang W, Lee PC, Jin JO. Time-dependent effect of *E. coli* LPS in spleen DC activation in vivo: Alteration of numbers expression of costimulatory molecules production of pro-inflammatory cytokines and presentation of antigens. Mol Immunol. 2017;85:205-213. DOI: 10.1016/j.molimm.2017.02.017
33. Du Sert, NP, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 2020;18:e3000411. DOI: 10.1371/journal.pbio.3000411
34. Xiao L, Morgan UM, Limor J, Escalante A, Arrowood M, Shulaw W, Thompson RC, Fayer R, Lal AA. Genetic diversity within *Cryptosporidium parvum* and related Cryptosporidium species. Appl Environ Microbiol. 1999;65:3386-3391. DOI: 10.1128/AEM.65.8.3386-3391.1999
35. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-275. [available at]
36. Lutz, MB, Kukutsch NA, Menges M, Rößner S, Schuler G. Culture of bone marrow cells in GM-CSF plus high doses of lipopolysaccharide generates exclusively immature dendritic cells which induce alloantigen-specific CD4 T cell anergy in vitro. Eur J Immunol. 2000;30:1048-1052. DOI: 10.1002/(sici)1521-4141(200004)30:4<1048::aid-immu1048>3.0.co;2-w
37. Connick K, Lalor R, Murphy A, O'Neill SM, El Shanawany EE. *Sarcocystis fusiformis* whole cyst antigen activates pro-inflammatory dendritic cells. J Parasit Dis. 2020;44:186-193. DOI: 10.1007/s12639-019-01181-9
38. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. Nat Rev Immunol. 2002;2:151. DOI: 10.1038/nri746
39. Ueno H, Palucka AK, Banchereau J. The expanding family of dendritic cell subsets. Nat Biotechnol. 2010;28:813. DOI: 10.1038/nbt0810-813
40. Ehigiator HE, McNair N, Mead JR. *Cryptosporidium Parvum*: The contribution of Th1-inducing pathways to the resolution of infection in mice. Exp Parasitol. 2007;115:107-13. DOI: 10.1016/j.exppara.2006.07.001
41. Urban JF, Fayer R, Chen SJ, Gause WC, Gately MK, Finkelman FD. IL-12 protects immunocompetent and immunodeficient neonatal mice against infection with *Cryptosporidium parvum*. J Immunol. 1996;156:263-268. [available at]
42. Pollok RC, Farthing MJ, Bajaj-Elliott M, Sanderson IR, McDonald V. Interferon-gamma induces enterocyte resistance against infection by the intracellular pathogen *Cryptosporidium parvum*. Gastroenterol. 2001;120:99-107. DOI: 10.1053/gast.2001.20907
43. Ehigiator HN, Mcnair N, Mead JR. IL-12 knockout C57BL/6 mice are protected from re-infection with *Cryptosporidium parvum* after the challenge. J Eukaryot Microbiol. 2003;50:539-542. DOI: 10.1111/j.1550-7408.2003.tb00622.x
44. Campbell LD, Stewart JN, Mead JR. Susceptibility to *Cryptosporidium parvum* infections in cytokine- and chemokine-receptor knockout mice. J Parasitol. 2002;88:1014-1016. DOI: 10.1645/0022-3395(2002)088((1014:STCPII)2.0.CO;2
45. Aggarwal S, Ghilardi N, Xie MH, De Sauvage FJ, Gurney AL. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J Biol Chem. 2003;278:1910-1914. DOI: 10.1074/jbc.M207577200
46. Diehl S, Rincón M. The two faces of IL-6 on Th1/Th2 differentiation. Mol Immunol. 2002;39:531-6. DOI: 10.1016/s0161-5890(02)00210-9
47. Robinson P, Okhuysen PC, Chappell CL, Lewis DE, Shahab I, Lahoti S, White AC. Expression of IL-15 and IL-4 in IFN-γ-independent control of experimental human *Cryptosporidium parvum* infection. Cytokine. 2001;15:39-46. DOI: 10.1006/cyto.2001.0888
48. Lacroix S, Mancassola R, Naciri M, Laurent F. *Cryptosporidium parvum*-specific mucosal immune response in C57BL/6 neonatal and gamma interferon-deficient mice: Role of tumor necrosis factor-alpha in protection. Infect immun. 2001;69:1635-1642. DOI: 10.1128/IAI.69.3.1635-1642.2001
49. Lean IS, Lacroix-Lamandé S, Laurent F, McDonald V. Role of tumor necrosis factor-alpha in development of immunity against *Cryptosporidium parvum* infection. Infect immun. 2006;74:4379-4382. DOI: 10.1128/IAI.00195-06
50. Couper K, Blount DG, Riley EM. IL-10: The master regulator of immunity to infection. J Immunol. 2008;180:5771-5777. DOI: 10.4049/jimmunol.180.9.5771
51. Perez-Cordon G, Yang G, Zhou B, Nie W, Li S, Shi L, Tzipori S, Feng H. Interaction of *Cryptosporidium parvum* with mouse dendritic cells leads to their activation and parasite transportation to mesenteric lymph nodes. Pathog Dis. 2014;70:17-27. DOI: 10.1111/2049-632X.12078
52. Bąska P, Zawistowska-Deniziak A, Norbury LJ, Wiśniewski M, Januszkiewicz K. *Fasciola hepatica* isolates induce different immune responses in unmaturated bovine macrophages. J Vet Res. 2019;63:63-70. DOI: 10.2478/jvetres-2019-0011
53. Chen XM, O'Hara SP, Nelson JB, Splinter PL, Small AJ, Tietz PS, Limper AH, LaRusso NF. Multiple TLRs are expressed in human cholangiocytes and mediate host epithelial defense responses to *Cryptosporidium parvum* via activation of NF-κB. J Immunol. 2005;175:7447-7456. DOI: 10.4049/jimmunol.175.11.7447
54. Schmidt W, Wahnschaffe U, Schäfer M, Zippel T, Arvand M, Meyerhans A, Riecken EO, Ullrich R. Rapid increase of mucosal CD4 T cells followed by clearance of intestinal cryptosporidiosis in an AIDS patient receiving highly active antiretroviral therapy. Gastroenterol. 2001;120:984-987. DOI: 10.1053/gast.2001.22557
55. Bedi B, McNair NN, Mead JR. Dendritic cells play a role in host susceptibility to *Cryptosporidium parvum* infection. Immunol Lett. 2014;158:42-51. DOI: 10.1016/j.imlet.2013.11.015

مستضد الأبواغ الخبيئة بارفم يحث على نضوج الخلايا الشجرية التي تثبط سايتوكينات الخلايا اللمفية التائية المساعد الثانية عند زرعها مع خلايا عنقود التمايز ٤

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الخلاصة

الأبواغ الخبيئة هو طفيل يسبب المرض في الحيوانات مثل العجول والماعز. ويعد أيضًا من الأمراض المشتركة التي تصيب الإنسان على مستوى العالم، ويسبب إسهالًا خفيفًا إلى شديد لدى الإنسان. في الحيوانات التي تعاني من نقص المناعة والعجول والحملان والبشر الذين يعانون من نقص المناعة مثل مرضى الإيدز، يمكن أن تكون العدوى مهددة للحياة حيث لا تتوفر حاليًا علاجات فعالة للسيطرة على العدوى. تم دراسة تأثير مستضد الأبواغ الخبيئة على الخلايا التشجرية. فحصت هذه الدراسة إفراز السيتوكين وتعبير علامة سطح الخلية على الخلايا التشجرية التي تم تعرضها ل CPA. تم أيضًا قياس إنتاج السيتوكين في خلايا CD4 + المزروعة بالاشتراك مع DCs معدة CPA في وجود مضاد CD3. تسبب CPA في زيادة كبيرة في إنتاج الإنترلوكين (IL-12p40 و IL-10 و IL-6 و TNF-α بواسطة DCs وعزز التعبير عن علامات سطح الخلية TLR4 و CD80 و CD86 و MHC11، .CPA معدة DC المزروعة بشكل مشترك في وجود مضاد CD3 مع CD4 + خلايا T تمنع إفراز السيتوكينات المرتبطة بـ Th2 ، ولا سيما IL-5 و IL-13 ، مع عدم وجود آثار على إفرازات الإنترفيرون (-IFN، IL-2 و IL-17 و IL-10. تدعم هذه النتائج الدراسات في الأدبيات التي تفيد بأن اتفاق مستضد الأبواغ الخبيئة يمكن أن يحفز النضج الكامل لمراكز البيانات التي تبدأ بعد ذلك الاستجابات المناعية Th1 الحاسمة لحل عدوى الأبواغ الخبيئة.

56. Aguirre SA, Mason PH, Perryman LE. Susceptibility of major histocompatibility complex (MHC) class I-and MHC class II-deficient mice to *Cryptosporidium parvum* infection. Infect Immune. 1994;62:697-699. DOI: 10.1128/iai.62.2.697-699.1994
57. Rahman M, Chapel H, Chapman RW, Collier JD. Cholangiocarcinoma complicating secondary sclerosing cholangitis from cryptosporidiosis in an adult patient with CD40 ligand deficiency: Case report and review of the literature. Int Arch Allergy Immunol. 2012;159(2):204-208. DOI: 10.1159/000337457
58. Mateen S, Zafar A, Moin S, Khan AQ, Zubair S. Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. Clin Chim Acta. 2016;455:161-171. DOI: 10.1016/j.cca.2016.02.01
59. Xu, QM, Fang, F, Wu, SH, Shi, ZQ, Liu, Z, Zhao, YJ, Zheng, HW, Lu, GX, Kong, HR, Wang, GJ, Ai, L, Chen, MX, Chen, JX. Dendritic cell TLR4 induces a Th1-type immune response against *Cryptosporidium parvum* infection. Trop Biomed. 2021;38:172-179. DOI: 10.47665/tb.38.1.029
60. El Shanawany EE, Nassar SA, Ata EB. Detection of humoral and cellular immune responses in buffaloes naturally infected with sarcocystosis with risk factor assessment. Acta Vet Beogr. 2019;69:275-89. DOI: 10.2478/acve-2019-0023