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Assessment of *Cryptosporidium parvum* infection in immunocompetent and immunocompromised mice and its role in triggering intestinal dysplasia

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SUMMARY

Objectives: There is an association between chronic inflammation and cancer, including colon cancer. *Cryptosporidium parvum* is a protozoan parasite that infects the gastrointestinal epithelial cells causing several parasitological and pathological changes. It is incriminated in the development of colorectal cancer in immunosuppressed individuals. Cyclin D1 expression is essential for cell cycle progression and its overexpression has been reported in colorectal cancer. This work aimed to study the gastrointestinal changes, including parasitological and pathological changes, induced by *C. parvum* infection in both immunocompetent and in chemically immunosuppressed mice, together with immunohistochemical assessment of cyclin D1 expression in infected tissues. In addition, the effectiveness of nitazoxanide (NTZ) in the treatment of cryptosporidiosis was evaluated.

Methods: This study included six groups of mice: group I, infected; group II, infected and immunosuppressed; group III, infected and treated with NTZ; group IV, infected, immunosuppressed, and treated with NTZ; and groups V and VI representing non-infected controls. Mice were subjected to stool examination for oocyst counts and were later sacrificed for intestinal dissection and routine histopathological examination of pathological changes; the endogenous developmental stages of the parasite were counted and immunohistochemical staining was carried out for the determination of cyclin D1.

Results: Group II showed the highest numbers of oocysts shed and endogenous developmental stages compared to the other groups. Intestinal dysplastic changes were seen only in groups I and II, where these changes were in favor of group II compared to group I. High-grade dysplasia was seen in four out of 20 mice in group II and was significantly associated with the number of endogenous developmental stages of *C. parvum*. NTZ was effective in the treatment of Cryptosporidium infection, with a greater effect in group III than in group IV.

Conclusions: *C. parvum* is one of the infectious agents that may induce intestinal dysplasia, including the high-grade category, which occurs particularly in the presence of immune suppression states and elevated endogenous parasite loads. Cyclin D1 is a good and useful marker for the detection of intestinal dysplasia. The effectiveness of NTZ is dependent on the immune status of the infected host.

1. Introduction

Cryptosporidium parvum is a globally distributed protozoan parasite that is found in both vertebrates and invertebrates.[1] Infections are transmitted by the fecal–oral route, or through contaminated food or water, and several major waterborne outbreaks have occurred.[2]

Cryptosporidiosis represents a major health problem as it is a frequent cause of diarrhea in both immunocompetent and immunodeficient individuals.[3] The risk of developing a severe disease differs depending on the personal immune status. In immunocompetent humans, exposure usually results in a self-limited disease manifested by watery diarrhea with a duration of about 2 weeks.[4] In contrast, in immunocompromised patients the parasite is of particular clinical significance, since it may cause a severe persistent disease that may be life-threatening.[5,6]

The immunocompromised population is a diverse group of individuals whose immune problems can result from an acquired immune deficiency syndrome (AIDS), cancer chemotherapy, or

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organ transplantation.[7,8] In addition, pregnant women, preschool children, and infants are more susceptible to the spread of this organism.[9]

C. parvum can be propagated in either chemically (steroids) or genetically immunosuppressed mice.[10] Generally, there is an association between chronic inflammation and cancer; colon cancer with inflammatory bowel disease is a common example of this association.[11]

C. parvum causes several pathological changes in gastrointestinal epithelial cells, especially in the immunocompromised host.[12] This parasite may result in a higher risk of developing colorectal malignancies, especially in those with severe immunosuppression.[13]

Cyclin Dl protein overexpression has been reported in colon carcinoma cell lines, in 30-46% of primary colorectal cancers,[14,15] and in at least 33% of sporadic colorectal adenomatous polyps in humans.[16] Cyclin Dl expression has been found to be essential for cell cycle progression in both non-transformed and transformed cells in which it is expressed, with inhibition of expression resulting in G1 growth arrest.[17]

In this work, cryptosporidiosis was studied in both immunocompetent and in chemically immunosuppressed mice (suppressed with dexamethasone). The intensity of oocyst shedding in the stool and histopathological changes in the gastrointestinal tract were examined, focusing on dysplastic changes and the grade of the dysplasia, together with the immunohistochemical evaluation of the role of cyclin D1 in highlighting these changes. In addition, examination of the effectiveness of nitazoxanide (NTZ) in the treatment of cryptosporidiosis was carried out, since no specific and effective therapy for this opportunistic infection is yet available.

2. Materials and methods

2.1. The animal

This study was carried out on laboratory bred Swiss albino female mice ($n = 80$) weighing approximately 20 g. They were all free from any parasitic infection on three consecutive days, as determined by examining their stools using the formol–ether concentration method[18] and modified Ziehl–Neelsen technique.[19]

The experimental animals were categorized into the following groups: (1) group I ($n = 20$), divided into two subgroups: Ia, 10 mice orally infected with Cryptosporidium oocysts and sacrificed on day 18 post-infection (PI), and Ib, 10 mice orally infected with Cryptosporidium oocysts and sacrificed on day 30 PI; (2) group II ($n = 20$), divided into two subgroups: IIa, 10 mice infected with Cryptosporidium oocysts, immunosuppressed using oral dexamethasone and sacrificed on day 18 PI, and IIb, formed of 10 mice infected with Cryptosporidium oocysts, immunosuppressed using oral dexamethasone and sacrificed on day 30 PI; (3) group III, formed of 10 infected mice, treated with the tested drug and sacrificed on day 30 PI; (4) group IV, formed of 10 infected mice, immunosuppressed using oral dexamethasone and then treated with the tested drug and sacrificed on day 30 PI; (5) group V, formed of 10 non-infected mice, immunosuppressed using oral dexamethasone (control group); (6) group VI, 10 non-infected mice (control group).

2.2. Immunosuppression

Immunosuppression was performed by giving the animals synthetic corticosteroids (dexamethasone) orally at a dose of 0.25 mg/g/day for 14 successive days prior to inoculation with Cryptosporidium oocysts.[20] The mice continued to receive dexamethasone at the same dose throughout the experiment.

2.3. The oocysts

Cryptosporidium oocysts were obtained from naturally infected calves (from slaughter houses) by collection of scrapings of the ileal mucous membrane and cecal content.[21] The samples were examined for confirmation of the presence of oocysts by modified Ziehl–Neelsen staining method.[19] The samples were then processed for DNA extraction and PCR amplification of *C. parvum*.[22] Genotypic classification was achieved using a portion of the 60 kDa glycoprotein gene (designated pgp60), which identified the different strain subtypes.[23,24] Subtype IIa A15G2R1 was selected because it is considered the prevailing subtype in calves and cattle in many different countries, including European countries and the USA.[25]

The infective samples were preserved by mixing with an equal volume of 2.5% potassium dichromate ($K_2Cr_2O_7$), in accordance with Current et al.[26] and Campbell and Current.[27] The infective inoculum was prepared in accordance with Reese et al.,[28] and the number of Cryptosporidium oocysts in the concentrated stock inoculums was determined using a hemocytometer.[29]

2.4. The infection

All mice in the studied groups except the control groups were infected orally with the prepared inoculums of Cryptosporidium oocysts; this occurred on day 15 of dexamethasone in the immunosuppressed groups.[30] The animals were deprived of water overnight, and were then inoculated intra-esophageally with the prepared inoculums using a tuberculin syringe connected to a polythene tube. The amount given to each mouse was adjusted to contain approximately 10^5 oocysts.[31]

2.5. The drugs

All mice in groups III and IV were treated orally with NTZ at a dose of 500 mg twice daily starting on day 14 PI: for three consecutive days for immunocompetent mice and for six consecutive days for immunosuppressed mice. The dose of the drug was calculated according to the Paget and Barnes table.[32] The drug was administrated to the mice using the same special syringes that were used for the oocyst inoculation.

2.6. Assessment of infection and the drug effect

2.6.1. Stool examination

Fresh fecal pellets from each mouse in the study groups were collected separately every 2 days over the 30 days of the experiment, according to the group to which they were assigned. Each sample was suspended in 10% formalin and homogenized. Then, 1 mg was prepared as a fecal smear and stained by the modified Ziehl–Neelsen staining method. The stained fecal smear was examined microscopically and the number of Cryptosporidium oocysts was counted in 10 high-power fields (HPF); the number of oocysts per mg for each animal and then for each group of animals was calculated. The mice in group Ia and group IIa (infected immunocompetent and infected immunosuppressed) were sacrificed first – on day 18 PI – in addition to five mice from each non-infected control group (groups V and VI). The remaining groups continued the experiment to day 30 PI, at which time fresh fecal pellets were collected, and the mice were then sacrificed.

2.6.2. Histopathological examination

The terminal 2 cm of the ileum and the liver were submitted to routine histopathological processing at the Pathology Department, Faculty of Medicine, Menofiya University, where they were fixed in 10% neutral buffered formalin, dehydrated in ascending grades of

ethanol, followed by immersion in xylene, and then impregnated in paraffin. Two 5-μm thick sections were taken from each block. One section was stained with hematoxylin and eosin for evaluation, as detailed below.

The severity of infection was assessed by counting the endogenous stages of the parasite in the epithelium in 10 villous crypt units; then the mean number per single villous crypt unit for each animal and for each group of animals was determined.[33]

The presence of a dysplastic epithelium was investigated, and when evident was classed as low-grade or high-grade in accordance with the World Health Organization criteria,[34] as follows: (1) low-grade dysplasia: slightly stratified spindle or oval nuclei with apical cytoplasm (roughly half of the total cell height) and evidence of goblet cell formation; (2) high-grade dysplasia: stratified, round nuclei and little cytoplasm (most nuclei reach the gland lumen) with greater nuclear pleomorphism, little goblet cell differentiation, reduced or absent cytoplasmic mucin production, and prominent mitotic figures.[35] This grade also showed architectural changes in the form of irregular branching, budding, or cribriform configurations of crypts.[36]

2.6.3. Immunohistochemical staining for cyclin D1

Other sections of the terminal ileum and liver were cut on superfrost slides, which were submitted to subsequent steps of deparaffinization, rehydration, blocking of endogenous peroxidase activity, and antigen retrieval by boiling in 10 mM citrate buffer, pH 6.0, for 10 min. The tissues were then incubated overnight at room temperature with primary antibody (at a dilution of 1:100; rat polyclonal antibody (Sc52893) raised against cyclin D1; Santa Cruz, USA). The detection kit was the UltraVision Detection System Anti-Polyvalent HRP/DAB (ready to use) (catalog number TP-015-HD; Lab Vision, California, USA). A chromogenic reaction was carried out with DAB substrate and the sections were counterstained with Mayer's hematoxylin. Mantle cell lymphoma was used as a positive control. Negative controls obtained by substitution of primary antibodies with rat-non immune serum were included in the staining procedure. Nuclear staining in any number of cells was required to assign cyclin D1 positivity.

2.7. Statistical analysis

Data were collected, tabulated, and statistically analyzed using SPSS program version 11. Fisher's exact test was used to compare qualitative variables, while the Student's *t*-test was used in comparisons between quantitative variables. A *p*-value equal to or less than 0.05 was considered significant.

3. Results

3.1. Oocyst shedding

Maximum shedding of *C. parvum* oocysts (Figure 1A) in immunocompetent mice (groups Ia and Ib) was observed on days 13 and 15 PI, with a mean of 3.2 ± 0.83, while in immunosuppressed mice (groups IIa and IIb), the mean number of oocysts shed in the stools on the same days was 4.8 ± 0.83; this difference was statistically significant ($p < 0.0001$). After NTZ treatment, infected immunocompetent mice (group III) showed a marked decrease in oocyst shedding, until it nearly ceased on day 21 PI in eight out of the 10 of mice in this group, while two of 10 continued to shed oocysts until day 30 PI. However, the mice in group Ib, which did not receive treatment, also showed a progressive decline in oocyst shedding, with almost complete cessation of oocyst production at the end of the experiment (day 30 PI). There was a difference between treated and untreated immunocompetent mice (groups III and Ib, respectively)

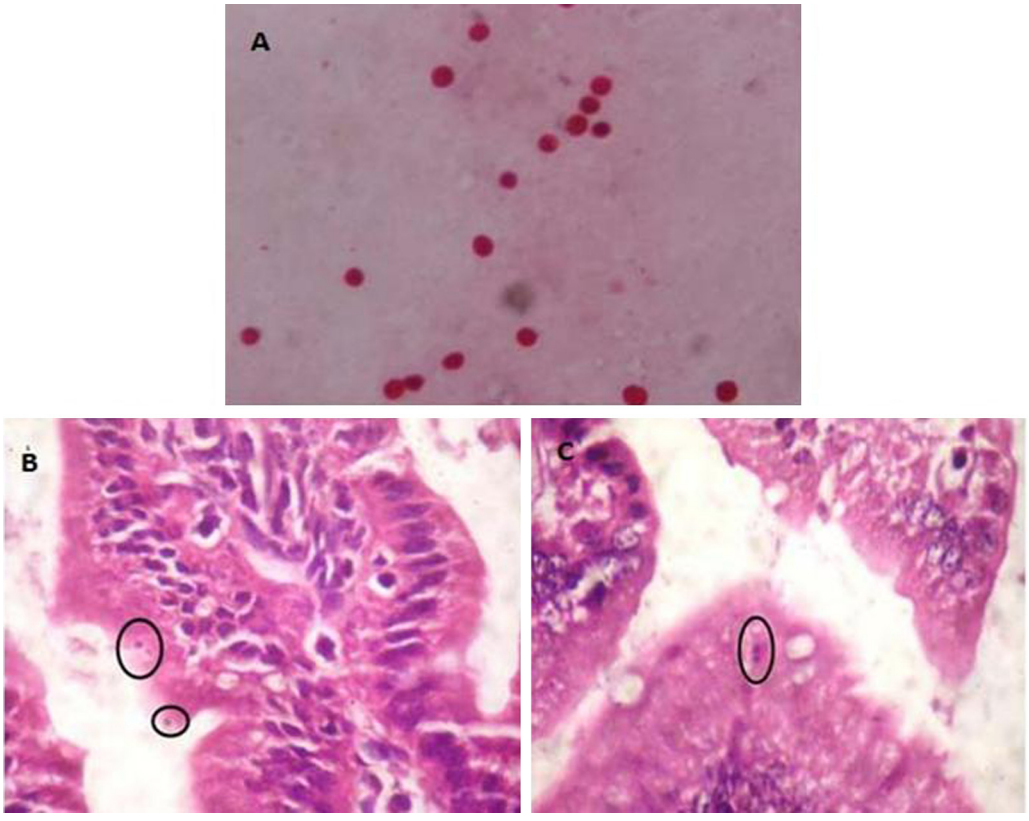

Figure 1. Cryptosporidium oocysts in the stool sample of an immunocompromised mouse (×1000) (A). The endogenous developmental stage of *Cryptosporidium parvum* (highlighted with circles) in the affected intestinal villi (hematoxylin and eosin staining ×600) (B and C).

Table 1
Comparisons between the different study groups with regard to the number of oocysts of *Cryptosporidium parvum* shed

Group	Shed oocysts/mg, mean ± SD	Time of stool examination	P1[a]	P2[a]	P3[a]	P4[a]
Group I	3.2 ± 0.83	15 days PI	T = 6.096	T = 0.94	T = 8.037	T = 3.91
Group Ib	1 ± 1	30 days PI	$p < 0.0001$	$p = 0.35$	$p < 0.0001$	$p = 0.001$
Group II	4.8 ± 0.83	15 days PI				
Group IIb	5.4 ± 1.14	30 days PI				
Group III	0.6 ± 0.89	30 days PI				
Group IV	2 ± 0.70	30 days PI				

PI, post infection; T, Student's *t*-test.
[a] P1 tests the difference between groups I and II; P2 tests the difference between groups Ib and III; P3 tests the difference between groups IIb and IV; P4 tests the difference between groups III and IV.

with regard to the shedding of oocysts, but it lacked significance ($p > 0.05$) (Table 1).

In infected immunosuppressed mice (group IV), NTZ caused irregular shedding of oocysts until it dropped to a mean of 2 ± 0.70 on the last day of the experiment. This was significantly higher than that of treated immunocompetent mice (group III, 0.6 ± 0.89; $p = 0.001$). Group IIa and group IIb included the infected immunosuppressed mice that did not receive treatment and these showed high levels of oocyst shedding throughout the study period with a mean of 4.8 ± 0.83 at day 15 PI in group IIa and 5.4 ± 1.14 in group IIb on the last day. Four mice in this group died before the end of the experiment. The difference between treated and untreated immunosuppressed mice (groups IV and IIb, respectively) was statistically significant ($p < 0.0001$). Groups V and VI representing control normal mice and control immunosuppressed mice showed no oocysts in their stools (Table 1).

3.2. Detection of endogenous developmental stages of cryptosporidiosis

The ileum was the site with the heaviest burden of intestinal cryptosporidiosis (Figure 1, B and C). In group Ia, the mean number of endogenous developmental stages of the parasite at the end of day 18 PI was 8.078 ± 2.5, while it was 12.91 ± 2.3 in group IIa; this difference was statistically significant ($p = 0.0003$). However, on day 30 PI it was observed that there was a significant decrease in the mean number of endogenous developmental stages of Cryptosporidium in group Ib (1.03 ± 1.04), with a significant difference compared to group Ia ($p < 0.0001$). In group IIb, it continued to increase to reach 15.63 ± 4.7 on day 30 PI, with a significant difference between group Ib and group IIb ($p < 0.0001$) (Table 2).

After receiving NTZ, it was found that the mean number of the endogenous developmental stages of the parasite in group III dropped markedly to 0.956 ± 0.49 on day 30 PI, while it was 3.29 ± 0.9 in group IV; this difference was highly significant ($p < 0.0001$). Also, there was a significant difference ($p < 0.0001$) in the mean number of endogenous developmental stages of Cryptosporidium on day 30 PI between group IIb (15.63 ± 4.7) and group IV (3.29 ± 0.9) (Table 2).

3.3. Histopathological changes

Several degrees of inflammatory changes were seen in the groups infected with the parasites, both immunocompetent and immunosuppressed. As regards dysplasia, most dysplastic changes were seen in group II (a and b), featuring low-grade dysplasia in most of the cases (16/20, 80%) (Figure 2A), while high-grade dysplasia was seen in four cases (40%) in group IIb (Figure 2, B and C) and one of these cases also showed large cell dysplasia of the liver (Figure 3, A and B). Low-grade dysplastic changes were also seen in four mice (40%) belonging to group Ib, with a significant difference compared to group II ($p < 0.0001$) (Table 3). No evidence of dysplasia was seen in the other groups and no frank carcinoma developed throughout the experiment (30 days). In group II, the development of high-grade dysplastic changes was significantly ($p < 0.05$) associated with the number of endogenous developmental stages of the parasite, since high-grade dysplasia was associated with a higher mean number (18.85 ± 2.6) compared to low-grade dysplasia (13.12 ± 3.165) (Table 4).

3.4. Immunohistochemical staining of cyclin D1

The dysplastic nuclei of both low- and high-grade were strongly stained for cyclin D1 (Figure 4, A and B).

4. Discussion

Cryptosporidium species are common causes of gastroenteritis associated with severe life-threatening illnesses among immunocompromised individuals.[37] Host cell immunity against cryptosporidiosis is mediated by both Th1 and Th2 responses.[38,39] Also, it has been found that there is an interferon gamma (IFN-γ) mucosal response with increased levels of interleukin 15 during cryptosporidiosis. IFN-γ has been reported to induce enterocyte resistance against *C. parvum*.[40,41]

In the current work, dexamethasone, a synthetic glucocorticoid, was used to induce chemical immunosuppression in the mice. Glucocorticoids are known to have an effect on the priming of the

Table 2
Comparisons between the different study groups with regard to the number of endogenous developmental stages of *Cryptosporidium parvum*

Group	Developmental stages/villous unit, mean ± SD	Day of sacrifice	P1[a]	P2[a]	P3[a]	P4[a]	P5[a]
Group Ia	8.078 ± 2.5	18 days PI	T = 8.32	T = 4.49	T = 9.59	T = 8.15	T = 7.19
Group Ib	1.03 ± 1.04	30 days PI	$p < 0.0001$	$p = 0.0003$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Group IIa	12.91 ± 2.3	18 days PI					
Group IIb	15.63 ± 4.7	30 days PI					
Group III	0.956 ± 0.49	30 days PI					
Group IV	3.29 ± 0.9	30 days PI					

PI, post infection; T, Student's *t*-test.
[a] P1 tests the difference between groups Ia and Ib; P2 tests the difference between groups Ia and IIa; P3 tests the difference between groups Ib and IIb; P4 tests the difference between groups IIb and IV; P5 tests the difference between groups III and IV.

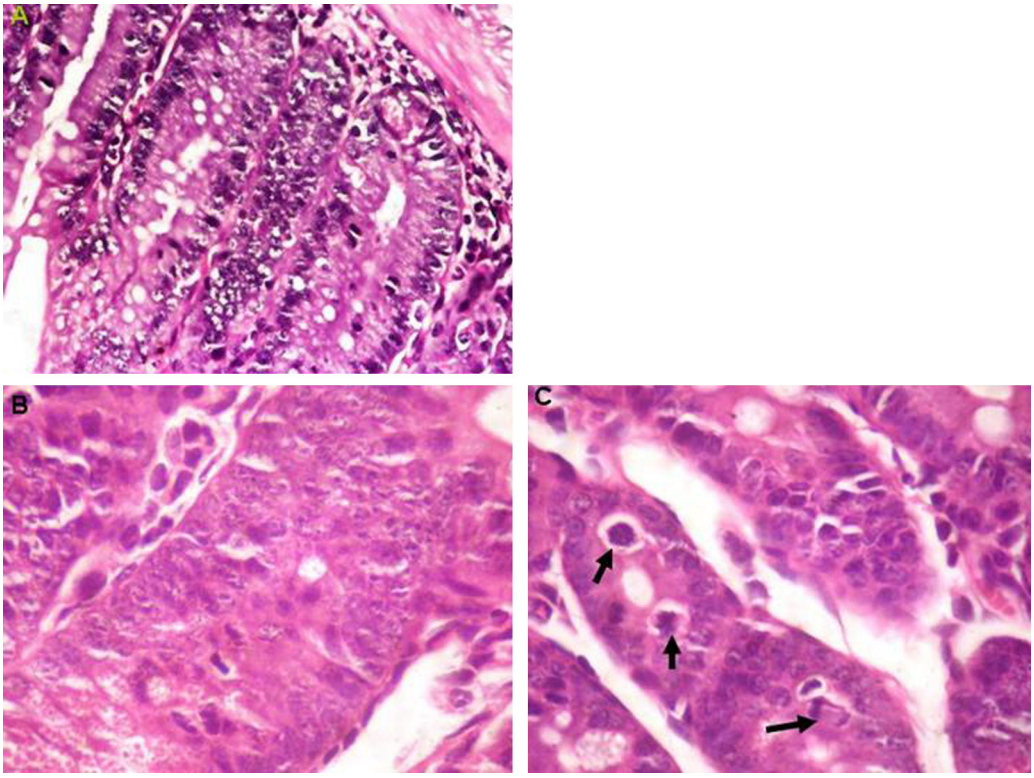

Figure 2. The affected intestinal epithelium showing low-grade dysplasia in the form of enlarged hyperchromatic nuclei that still showed apical cytoplasm (hematoxylin and eosin staining ×400) (A). Intestinal epithelium showing high-grade dysplasia manifested by great pleomorphism, absence of mucin, and frequent mitoses (arrows) (hematoxylin and eosin staining ×600) (B and C).

innate immune response[42] and could suppress IFN-γ-regulated expression.[43]

This study was carried out over a period of 30 days to evaluate the course of infection. Matsui et al.[44] reported that the interval which covers the natural shedding period of Cryptosporidium infection in mice is about 24 days; Lacroix et al.[12] found that the duration of oocyst shedding was about 3–4 weeks.The intensity of oocyst shedding was significantly higher in dexamethasone immunosuppressed mice than in immunocompetent ones throughout the duration of the experiment. Similar results have been reported by Kapel et al.,[45] Chai et al.,[46] and Certad et al.[13] The maximum shedding of oocysts in immunocompetent mice (groups Ia and Ib) was observed on days 13 and 15 PI, in agreement with Miller et al.[9] and Certad et al.,[13] and for the majority no oocysts were detected in the stool at the end of the experiment (group Ib). On the other hand, dexamethasone immunosuppressed mice (group IIa and IIb) showed high levels of oocyst shedding throughout and at the end of the experiment (group IIb). These results are in agreement with those of several studies.[13,47–49]

Regarding the histopathological findings, the terminal part of the ileum was found to be the site with the heaviest burden of infection in both immunocompetent and immunosuppressed mice, in agreement with the findings of others.[8,41,45,50] It has been suggested that conditions in the ileum are favorable, including biochemical conditions and the presence of specific receptors, and contribute to the development of the parasite.[51] Also, Certad et al.[13] found that parasitic localization and histopathological changes were mainly localized to the ileocecal region. The dysplastic changes observed in the current study were mainly of low-grade category, seen in 40% of group Ib and 80% of groups IIa and IIb, with only four cases (40%) belonging to group IIb showing high-grade dysplasia. The development of adenoma and intraepithelial neoplasia (synonymous with dysplasia) has also been reported by others using dexamethasone in treated adult severe combined immunodeficiency (SCID) mice.[13] In the present study, no frank carcinoma was detected, while Certad et al.[52] observed the development of intramucosal carcinoma with a suspicion of submucosal invasion in four mice in their experiment. The absence of frank carcinoma in our work could be related to the degree of immunosuppression used, which relied on dexamethasone; other studies used the SCID mouse model in addition to dexamethasone.[13,51] As the degree of immunosuppression increased, the risk of neoplastic transformation increased. In addition, they used different strains of Cryptosporidium, such as *C. parvum* IOWA and *C. parvum* TUM1, as in Certad et al.[52] According to the latter study, *C. parvum* TUM1 strain seemed to induce a more severe illness than *C. parvum* IOWA, with an earlier onset of neoplastic lesions that rapidly progressed to invasive cancer on day 20 PI.[52]

Although the present results showed mostly low-grade dysplasia induced by *C. parvum*, these changes still represent a potential precursor to digestive carcinoma. The prevalence of *C. parvum* in patients with colorectal cancer reached 18%, primarily in those with tumors located on the left side (in the sigmoid and descending colon). Furthermore, an epidemiological study performed in Poland reported a high frequency of cryptosporidiosis in patients with colorectal cancer.[53]

In the current study, a significant correlation was found between the development of high-grade dysplasia and the number of endogenous developmental stages of the parasite ($p < 0.05$). This reflects the impact of parasite load on neoplastic transformation. According to Certad et al.,[52] histopathological evidence of dysplasia in any organ was always associated with the presence of the parasite, and an elevated parasite load was correlated with the severity of ileocecal pathological changes.

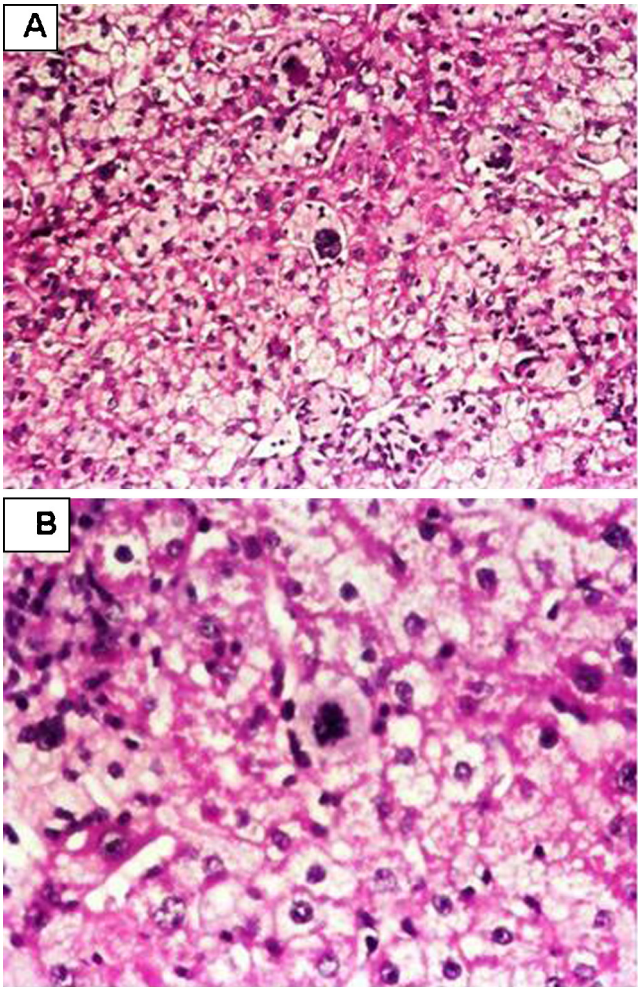

Figure 3. Large cell dysplasia of the liver in one of the infected mice manifested by irregular and abnormal hyperchromatic nuclei (hematoxylin and eosin staining, ×200 (A) and ×400 (B)).

Extra-intestinal dissemination of the parasite has also been reported with evidence of dysplastic changes in the bile duct, for example.[52] In the current study, there was one case demonstrating large cell dysplasia in the liver, which was concomitantly associated with high-grade ileal dysplasia; we believe that such an observation has not been reported previously.

Since reactive atypical changes secondary to inflammation and infection could morphologically affect the epithelium of the gastrointestinal tract and simulate true dysplastic changes, determining the true dysplastic process using specific markers is required. In the present study, the use of one cell cycle regulator – cyclin D1 – helped to identify true dysplasia. As a nuclear marker, its evaluation is reproducible, and we found it to stain the nuclei in dysplastic areas of both low- and high-grade categories. However, cyclin D1 did not stain normal areas; these findings agree with others.[54,55] In the latter study, cyclin D1 was detected in the intestinal adenomas of familial adenomatous polyposis syndrome and was associated with proliferative activity and the severity of dysplasia in these premalignant lesions. The authors suggested that abnormal upregulation of cyclin D1 may be an early event in intestinal carcinogenesis.[55]

Table 3
Dysplastic changes in the different study groups[a]

Groups	Group Ia	Group Ib	Group IIa	Group IIb	Group III	Group IV
Dysplasia						
Positive	0	4	6	10	0	0
Negative	10	6	4	0	20	20

[a] Ia and Ib versus IIa and IIb, Fisher's exact test $p = 0.0004$.

Table 4
The relationship between grade of dysplasia and the number of endogenous developmental stages of *Cryptosporidium parvum* in group II

	Low-grade dysplasia ($n = 16$)	High-grade dysplasia ($n = 4$)	Test
Developmental stage, mean ± SD	13.12 ± 3.165	18.85 ± 2.6	T = 2.337 $p = 0.04$

T, Student's *t*-test.

The endogenous developmental stages of the parasite were detected in sections obtained from immunocompetent mice at a high level on day 18 PI (group Ia). However on the last day of the experiment (day 30 PI), few were detected in histological sections (group Ib, Table 2). These findings are in agreement with Lacroix et al.[12] and Takeuchi et al.,[56] who found that the immune system and defense mechanisms are able to fight the infection and reject the parasite rapidly in the immunocompetent host. On the other hand, the endogenous developmental stages of the parasite were detected in histological sections obtained from dexamethasone-immunosuppressed mice in both group IIa and group IIb with a statistically significant difference in comparison to the other groups of immunocompetent mice (Table 2). These results could be attributed to the suppression of the immune system by dexamethasone, making clearance of Cryptosporidium organisms very difficult, which then increased the severity and duration of infection in immunosuppressed mice. The findings of several studies suggest that the mucosal immune defenses play a major role in preventing Cryptosporidium infection and that the common factor in immunosuppressed mice is the suppression of lymphocytes that leads to chronic infection.[41,57,58]

Many compounds have been tested for their potential anti-cryptosporidial activity, but there is no efficient therapy to prevent or treat cryptosporidiosis, particularly in the immunocompromised host.[5,59] The parasite's unique location in the host cell may affect the drug concentration, and the existence of transport proteins or efflux pumps that transport drugs out of the parasite or into the host cells provides the Cryptosporidium organisms with resistance to drug therapy. This explains why many therapies are not effective against the parasite.

NTZ and other nitrothiazole salicylamide compounds have broad antiparasitic activities.[60] In the present study, NTZ was tested as a treatment for cryptosporidiosis in both the experimentally infected immunocompetent and dexamethasone-immunosuppressed groups (group III and group IV, respectively). Our study demonstrated the effectiveness of NTZ in both groups with significant differences regarding levels of oocyst excretion in the stool and the number of endogenous developmental stages of the parasite in both groups, being lower in group III (immunocompetent mice) than in group IV ($p < 0.05$) (Tables 1 and 2). These results agree with those of Bailey and Erramouspe[61] and Fox and Saravolatz,[62] who found that the responses to the drug were lower in immunocompromised individuals. Also, Gargala[63] found that NTZ significantly shortened the duration of diarrhea and decreased mortality in adults and in malnourished children. However, the latter study showed that NTZ is not effective without an appropriate immune response, in which a competent immune system is needed to reject the parasite. Rossignol reported the variability of the effectiveness of NTZ, which depended on the degree of immunosuppression and CD4 counts.[3]

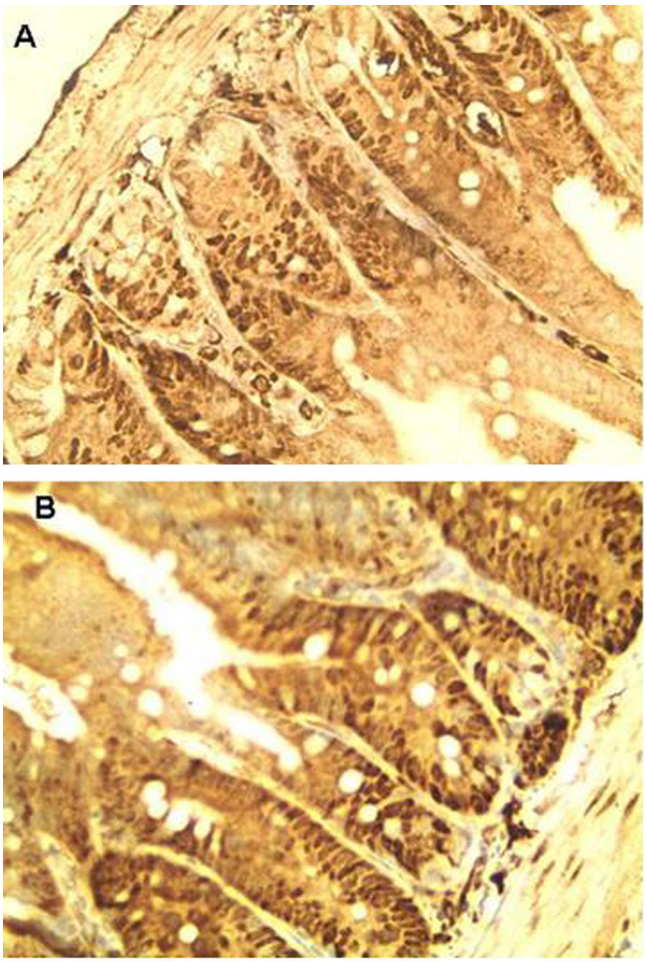

Figure 4. Nuclear expression of cyclin D1 in the dysplastic intestinal epithelium infected by *Cryptosporidium parvum* (immunohistochemical staining, ×100 (A) and ×200 (B)).

Although, the drug caused a marked decrease in the mean number of oocysts per milligram after the initiation of therapy in group III, the results showed no significant difference ($p < 0.05$) when compared to group Ib, and no oocysts were detected in the stools of either group by the end of the experiment. It is clear that the competent immune system rejected the parasite and that it caused a self-limited disease in the untreated group, while infected untreated immunosuppressed mice (group IIb) still showed a high level of oocyst excretion until the last day of the experiment (Table 1). This may be due to a failure of the immune system to eradicate the parasite. Similar findings have been recorded in several studies including in vitro and in vivo studies using several animal models and in clinical trials, which have demonstrated the effectiveness of NTZ in treating diarrhea and enteritis caused by *Cryptosporidium* species in immunocompetent patients.[61,64] On the other hand, Amadi et al.[65] found no significant effect of NTZ on the eradication of cryptosporidial infections in Zambian children with HIV-related immunosuppression.

In conclusion, *Cryptosporidium parvum* is one of the infectious agents that may induce intestinal dysplasia, even of high-grade category, which is highly affected by immune suppression states and elevated endogenous parasite loads. Cyclin D1 is a good and useful marker for the detection of intestinal dysplasia. The effectiveness of NTZ is dependent on the immune status of the infected host.

Conflict of interest: No conflict of interest to declare.

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