Immune response of newborn BALB/c mice to *Cryptosporidium* infection

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Abstract *Cryptosporidium parvum* is a protozoan parasite which causes diarrheal in human and animals worldwide. Infection transmission has reported through oral-fecal by infectious objects through foods and drinks. In this study we explored the immune response pathway in animal model for *C. parvum* to develop the new treatment way. Oocysts collected from fecal positive for *C. parvum* and diluted about 1:5 in sucrose solution. New born BALB/c mice (3 days) divided to 2 different groups. Control group hadn't received any oocyst, the test groups received 5×10^5 oocysts. 5 mice selected for each control group and 11 mice chosen for each test group. Blood collected from heart bleeds in days of 6, 9, 12 and 16. Protein concentrations determined by bio-photometer. Dot blotting used to find out total antibody concentrations oocyst antigen. Among the test and the control groups, blots appeared in test group which means antibody production, but not any

blot observed in the control groups. The non-characteristic proteins in serum were measured by the biophotometer. In this study, we investigated antibody serum production against *C. parvum* oocysts in new born BALB/c mice. The detected antibody through dot blot technique was our aims which had conjugated to our characteristic antiserum. The recorded numbers for the controls by biophotometer related to the non characteristic proteins in serum. The results of this study can used to produce polyclonal or monoclonal antibodies against cryptosporidiosis.

Keywords *Cryptosporidium parvum* · BALB/c mice · Immune response

Introduction

Cryptosporidium parvum is an intracellular and unicellular protozoan parasite that causes cryptosporidiosis disease (McDonald et al. 2004; Bouzid et al. 2013). The parasite begins its life cycle through the intake of oocytes by the host infection transmission through oral-fecal directly or indirectly contact with infectious objects throughout foods and drinks (Kothavade 2011; McDonald et al. 2013; Current et al. 1986). *Cryptosporidium* oocytes have high resistant to most frequent disinfectants, and survive for a month in humid and cold condition (Mirza-Qavami and Sadraei 2011). Cryptosporidiosis is to infect mammalian intestinal tract with a watery diarrhea (Leitch and He 2012), while the most cases of *Cryptosporidium parvum* infections are asymptomatic. Cryptosporidiosis often associated with fever, vomiting, diarrhea, nausea, and abdominal discomfort, that it takes 2 weeks to resolve (Leitch and He 2012). Despite the more intense investigations, cryptosporidiosis remains one of the most common

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infections in the nations, mainly in newborns and young children with malnutrition symptom in the third world countries (McDonald et al. 2013).

The immune system of person has an important role in infection with the parasite. Immune-competent hosts have ability to limit cryptosporidiosis but in immune-suppressed hosts such as HIV/AIDS, it causes the most severe symptoms and may causes potentially fatal complications such as bile duct damage (Leitch and He 2012; Angus 1990; Manabe et al. 1998).

The parasite may promote apoptosis in beside epithelial cells while inhibiting apoptosis in the infected cells, promoting prolonged survival of the parasite. Because of the “minimally invasive” nature of *Cryptosporidium* infection, mucosal epithelial cells are critical to the host’s anti-*Cryptosporidium* immunity. Epithelial cells not only provide the first and rapid defense against *Cryptosporidium* infection, but also mobilize immune effector cells to the infection site to activate adaptive immunity. Pathogen recognition receptors (e.g., Toll-like receptors) in epithelial cells recognize *Cryptosporidium* and initiate downstream signaling pathways (e.g., NF-kappaB) which trigger a series of antimicrobial responses and activate adaptive immunity (Lüder et al. 2001).

Both humoral and cellular immunity play a role in the control of this infection, but the latter plays the major role, mainly in the intestinal mucosa. The capacity to produce all Th1, Th2 and Th17 cytokines, rather than the presence of Th2 cytokines alone, determines the effective immune response against *C. parvum* infection (Mead 2014).

Immune response to cryptosporidiosis related to innate and adaptive immune systems. Polyclonal antibody is one way to infection therapy. The AIDS patients in advanced stage with severe chronic diarrhea had treated with polyclonal antibodies. After 21 days of treatment diarrhea was decreased in all patients, but all of them remained infected (McDonald 2011; Greenberg and Cello 1996).

To conquer to *Cryptosporidium*, in this study we have found out the immune response of newborn BALB/c mice by *Cryptosporidium* oocyst infection to develop the new way for cryptosporidiosis disease treatment. For this aim we have measured the total antibody expression by dot blotting assay.

Materials and methods

Oocyst preparation and purification

Fecal samples positive for *Cryptosporidium parvum* obtained from naturally infected calves in Shahriar-Tehran, Iran. The feces diluted about 1:5 in sucrose solution (SG: 1.27) and centrifuged at $2,000\times g$ for 15 min. After centrifugation, the supernatant containing oocyst discarded and collected (Arrowood and Sterling 1987).

The study mice

New born BALB/c mice (3 days) used for all experimental procedures. The mice prepared from Razi Vaccine and Serum Research Institute (Hessarak, Karaj, Iran) and housed in stainless steel cages containing sterilized wood shavings. Pelleted food and water provided for them. The mice divided to 2 different groups. Control group hadn’t received any oocyst, the test groups received 5×10^5 oocyst. 5 mice selected for each control group and 11 mice chosen for each test group.

Immunization

The oocyst numbers counted by microscope and 5×10^5 (20 μ l) of them diluted with 2.5 ml PBS and gavaged to BALB/c mouse. After 6 days, oocyst present confirmed by microscope.

Mouse serum total antibody levels

Blood collected from heart bleeds in day of 6, 9, 12 and 16. Then they centrifuged at $5,000\times g$ for 5 min and the top layer of serum extracted carefully and stored at -20°C .

Oocyst antigen preparation

5×10^6 oocytes (200 μ l) suspended in lysis buffer (50 mM Tris pH 8, 10 % glycerol and 0.1 % Triton X-100) and sonicated 3 times for 20 s.

Protein concentration

Protein concentration determined by bio-photometer (Eppendorf).

Dot blotting

To find out total antibody concentrations oocyst antigen prepared as described in above. 3 μ l of antigen placed in nitrocellulose membrane and fixed through using UV light, then washed two times in TBS (10 mM Tris, pH 8 and 150 mM NaCl) for 10 min. After the membrane soaked in blocking solution (3 % BSA plus TBS). Following an hour it washed in TBS and incubated with primary antibody (serum).

Results

After the appreciate times 6, 9, 12 and 16 days, total protein concentration increased significantly rather than control group. The experiments showed the total protein

concentration (antibody) of BALB/c mice has significantly increased after 16th day of oocyst treatment (Table 1; Figs. 1 and 2).

Discussion

Antibodies recognizing parasite antigens may developed following infection or can induced by vaccination with parasite antigens, providing tools for experimental studies in immunology or therapy. During infection antigen-specific antibodies can detected in serum, including IgG, IgA and IgM. The IgM titers usually decline soon after infection brought under control while IgG may persist for several months at least. However, antibody titers may persist in children in developing countries, possibly because of continuous exposure to reinfection in a contaminated environment (McDonald 2011).

Cryptosporidium spp. infects the gastrointestinal epithelium of vertebrate hosts. Intestinal species typically cause self-limiting diarrhea in immunocompetent individuals, suggesting an efficient host immune defense to eliminate the infection. Both innate and adaptive immunity involved in host anti-parasite defense. Because of the “minimally invasive” nature of *Cryptosporidium* infection, mucosal epithelial cells are critical to the host’s anti-*Cryptosporidium* immunity. Epithelial cells not only provide the first and rapid defense against *Cryptosporidium* infection, but also mobilize immune effector cells to the infection site to activate adaptive immunity (Borad and Ward 2010). Attachment to the apical cell surface by *Cryptosporidium*, as well as molecules inserted into host cells after attachment, can activate host cell signal pathways and thereby alter cell function. Pathogen recognition receptors (e.g., Toll-like receptors) in epithelial cells recognize *Cryptosporidium* and initiate downstream signaling

Fig. 1 Protein concentration (mg/ml) for test groups after 6, 9, 12 and 16 days. PC protein concentration

pathways (e.g., NF-kappaB) which trigger a series of antimicrobial responses and activate adaptive immunity. Non-coding RNAs are critical regulators of mucosal immunity to infection, while release of exosomes from epithelial cells may be a relatively unexplored, important part of mucosal anti-parasite defense. Conversely, it appears that *Cryptosporidium* has also developed strategies of immune evasion to escape host immunity, at least at the early stage of infection (Chen et al. 2005).

Cryptosporidial infection leads to a parasite-specific antibody response in both serum and intestine but murine studies suggest that B cells are not essential for recovery from infection. Epidemiological studies suggest colostrum or parasite-specific colostral antibodies may provide a degree of passive immunity to suckling human offspring, but results from experimental studies with animals suggest previously infected dams do not protect offspring (Cox et al. 2005). There is evidence that a degree of passive immunity can obtained with orally administered colostral antibodies from dams immunized mucosally with crude or recombinant oocyst antigens and adjuvant or DNA plasmids encoding parasite antigens. Whether this approach to

Table 1 Protein concentration (mg/ml) in control and test groups after 6, 9, 12 and 16 days

PC group 1		PC group 2		PC group 3		PC group 4	
Control	Test	Control	Test	Control	Test	Control	Test
45.8	1,656.8	47.4	1,776.5	51.7	1,835.1	59.3	1,921.7
44.12	1,949.7	46.1	1,951.1	53.8	1,967.2	58.6	1,819.1
45.2	1,340.0	47.5	1,455.0	52.4	1,461.54	54.8	1,575.0
47.6	1,061.09	48.1	1,069.0	55.3	1,166.4	57.1	1,261.3
59.3	1,432.1	61.1	1,551.2	59.7	1,576.0	60.7	1,695.2
	1,967.8		1,991.2		1,996.1		1,998.7
	1,511.0		1,721.1		1,831.7		1,956.2
	2,529.3		2,651.4		2,744.1		2,865.6
	2,018.7		2,155.3		2,214.5		2,173.0
	1,467.5		1,692.8		1,671.0		1,756.4

PC protein concentration

Fig. 2 Dot blotting assay for determination of sensitivity of the antigen **a** protein extracted from mice after 6 days, **b1** after 9 days, **b2** after 12 days, **c** after 16 days of infection. Antigen was applied as 3 µl dots on a nitrocellulose strip

immunotherapy can efficiently scaled up and be cost effective remains to be seen (Frost et al. 2005; McDonald et al. 1992). The AIDS patients in advanced stage with severe chronic diarrhea had treated with polyclonal antibodies. After 21 days of treatment diarrhea was decreased in all patients, but all of them remained infected (Ungar et al. 1990).

This study designed to evaluate antibody production in infected newborn mice. The results showed the rate of antibody production has increased 6th day of the infection and maximized in the sixteenth day after infection. For to produce polyclonal antibodies is necessary to know the time of maximum production of effective antibody. The results of this study can be used to produce polyclonal or monoclonal antibodies against cryptosporidiosis.

Because there is no suitable treatment for cryptosporidial infection, using of polyclonal antibodies can be an effective way to improve the symptoms of the infection.

Finally, finding out passive immunity by antibody therapy has shown some promise in experimental studies. The antibodies would most likely have to be of high avidity and directed against antigens involved in sporozoite/merozoite attachment to host cells or in the host-cell invasion mechanism. Treatment based on colostrum or colostrum-derived antibodies from immunized animals might be practicable on a small scale. Larger scale commercial production would likely require monoclonal antibodies or antibody fragments. Oral administration of antibodies is desirable and the antibodies might need to be protected from degradation until they reach the site of infection, perhaps by encapsulation.

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