



Immunopathological Assessment of *Hydatid* Cyst and *Cysticercus Tenuicollis* Sonicated Protoscoiles Antigens in Mice

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ABSTRACT

The present study was designed to investigate the cross-protection (protective immunity) between Hydatid cyst and Bladder worm and evaluate the immunologic response of both humerol and cellular immunity in mice. To achieve these goals, 120 mice were used and equally divided into four groups immunized subcutaneously with 2 doses of antigen at the first and 14 days of the experiment. Mice in the first group (n=30) were immunized with 0.3 ml of hydatid cyst sonicated protoscolex antigen. Those in the second group (n=30) were immunized s/c with 0.3 ml of *Cysticercus tenuicollis* sonicated protoscolex antigen. The third group (n=30) was immunized with 0.3 ml of both antigens (0.15 + 0.15), and the fourth group was a control group in which the mice were intraperitoneally injected with 0.2 ml of phosphate buffer solution. At the end of the experiment (30 days), blood samples were taken from the hearts of mice in all groups after being anesthetized by intramuscular injection of Ketamine 60 mg/kg, and Xylazine 12 mg/kg for the assessment of mouse Interleukin-12, IgG, and tumor necrosis alpha levels. The skin test results 24 hours (day 28) post-immunization showed an increase in the skin thickness against both antigens in the treatments, compared to the control. However, there was a decrease at 48 hours (day 29) post-examination in all groups. The results of TNF α titer showed higher titer in the third group, compared to the first, second, and fourth groups. Interleukin 12 concentration showed a higher titer in the third group than in the first, second, and fourth groups. The IgG concentration showed higher titer in the third group compared to the first, second, and fourth groups. In conclusion, immunopathological studies have shown that Ags used in the study, induce humoral and cellular immunity, compared to each Ag alone, and the mixed antigens were much more immunogenic. This cross-reactivity and synergistic interactions between the two parasites may be the cause of their antigenic activities.

Keywords: Immunopathological cross reaction, Interlukin-12, Parasitic antigens, Sonicated protoscolex antigens, TNF α

INTRODUCTION

Both *Taenia hydatigena* cysticercosis and echinococcosis caused by *Echinococcus granulosus*, also known as hydatidosis, are common parasitic diseases that affect many wild and domestic animals as well as humans (Gessese 2020). They are regarded as one of the major causes of economic losses and livestock productivity in both the developing and industrialized worlds (Oryan et al., 2012). Proglottids or eggs carried in dog feces that pollute the pasture or feeding areas cause the intermediate host to become infected (Murell, 2005). Some canids, including wolves, jackals, and foxes, are natural hosts for cysts and can spread them (Parija, 2004). The loss is related to the condemnation of organs induced by hydatid and *Cysticercus tenuicollis* cysts in small ruminants. *Cysticercus tenuicollis*, a cystic development made up of many liquid-filled cysts that are specifically found on the fascia of the abdominal organs of ruminants, harbors the invaginated scolex of the future tapeworm. Generally, the causative organism is subclinically and mildly infected (Torgerson et al., 2008).

A hydatid cyst infection can produce T helper 1 and T helper 2 (cytokines). In echinococcosis, Th 1 cytokines are linked to a protective response, but Th 2 cytokines induce susceptibility to the disease. The immune response will promote parasite proliferation and development if cytokine response skews Th 1/Th 2 ratios in favor of a preferred immunopathology-associated Th 2 polarization. Additionally, cysts' illness, formation, and development are linked to (or indicated by) the serum antibody response (Zhang and McManus, 2008). It has been demonstrated that lambs

ORIGINAL ARTICLE
 pii: S2322-45682200048-12
 Received: 05 October 2022
 Accepted: 27 November 2022

generate both IgG1 and IgG2 as the primary immunoglobulins after exposure to *Cysticercus tenuicollis* infection (Craig and Rickard, 1982). IgG2 has a lower magnitude than IgG1, but it has a much more pronounced effect in terms of preventing infection. The main immunoglobulin subclass present in ruminant colostrums and milk is IgG. Many IgG subclasses, like IgG1, serve as the primary immunoglobulin in colostrums (Hurley and Theil, 2011).

The role of antibodies in the protection of sheep infection against several Taeniid metacestodes was examined using the passive transfer of immunoglobulin. In *Cysticercus tenuicollis*, a 70-80% reduction in cyst numbers was achieved by transferring 100-120 ml of serum from immunized sheep with *Cysticercus tenuicollis* oncospheres to recipients (Jacobs *et al.*, 1994). Cross-reactivity with different cestodes is seen in parasites due to the high number of shared antigens (El-Moghazy and Abdel- Rahman 2012). Numerous physical and antigenic similarities exist among different cestodes. *T. hydatigena* was employed as a model organism for *Taenia saginata*, *Echinococcus granulosus*, and other cestodes (Mcmanus, 2014; Miquel *et al.*, 2015).

Crude protein from the fluid of *Taenia hydatigena* cysts was employed as an ELISA antigen to detect antibodies against *Taenia saginata* cysticercosis, *Echinococcus granulosus* hydatidosis, and *Taenia solium* cysticercosis (Kamanga-Sollo *et al.*, 1987; Rhoads *et al.*, 1991 Bogh *et al.*, 1995; Kara *et al.*, 2003). Additionally, antigens can lead to immune-mediated cross-protection in the intermediate host for infections with *Cysticercus ovis*, *Cysticercus bovis*, *Cysticercus cellulose*, and Hydatid cyst. The development of metacestodes in these infections can be reduced by pre-exposure to *C. tenuicollis* (Conlan *et al.*, 2012). Thus, this study aimed to investigate the cross-protection (protective immunity) between hydatid cyst and bladder worm and evaluate the immunologic response of both humeral and cellular immunity in mice.

MATERIALS AND METHODS

Ethical approval

All experiences were approved by the ethical committee at the College of Veterinary Medicine, Kerbala, Iraq (ethical approval number: COVM-6341).

Study design

Hydatid cyst were collected randomly during inspection of the sheep carcasses in the abattoirs (Kerbala, Iraq) and transferred into the laboratory (Veterinary Medicine College in Kerbalaa, Iraq) for examination, by a cooling box. Hydatid protoscolices antigen was prepared following the method of Nasrieh and Abdel-Hafez (2004) with certain modifications. Collected hydatid fluid was clarified by centrifugation at 10000 rpm at 4°C for 60 minutes. The deposit containing protoscolices of hydatid cysts was washed thoroughly in phosphate buffer saline (PBS) three times to remove cyst wall debris and dead protoscolices. About 1.8 g of the washed pellet was suspended in 3 ml of PBS in which 40 µl of 0.2 molar phenyl methyl sulphonyl Fluoride (Sigma, USA) in isopropanol was added. The mixture was homogenized using a glass homogenizer (B-Braun Biotech International, Germany) for 20-30 strokes. The homogenate was sonicated at 50 cycles /s at the maximum tune of 1.8 µm peak to peak for 30 seconds four times in an ice bath using an ultra Sonicator (B-Braun Biotech International, Germany). The sonicate was centrifuged at 15,000 rpm at 4°C for 20 minutes. The supernatant was used as protoscolices antigen of hydatid protoscolex sonicated antigen (HPSA) and stored at -20°C. Similarly, *Cysticercus tenuicollis* scolex antigen (CTSA) was also prepared.

Experimental design

A total of 120 mice of both sexes (80 males and 40 females separated), aged from 4-6 weeks with 22 g mean weight were obtained from the Iraqi Center for Cancer and Medical Research, they were adopted at the animal house of Veterinary Medicine College in Baghdad, Iraq for 2 weeks before starting the experiment. The mice were divided into four groups with three replicates (10 mice in each replicate). Each group received 2 doses of the antigen for 14 days. In the first group, mice were immunized s/c with 0.3 ml of *Hydatid cyst* sonicated protoscolex Ag. In second group mice were immunized s/c with 0.3 ml of *Cysticercus tenuicollis* sonicated protoscolex Ag. In the Third group, mice were immunized s/c with 0.3 ml of both Ag, and in the fourth group mice were injected intraperitoneal with 0.2 ml PBS as the control negative group. Delayed-type hypersensitivity test (Skin test) was performed according to Jacysyn *et al.* (2003) and Silva *et al.* (2021) on mice groups after immunization. For all groups, the left hind footpad was injected with 0.1 ml sterile PBS. The thickness of the skin was measured by a vernier caliper on day 28, and day 29 of the experiment

Blood samples were taken one-time from the hearts of the mouse groups after anesthetized rats by intramuscular injection of Ketamine 60 mg/kg and Xylazine 12 mg/kg, at the end of the experiment (30 days). The blood samples were centrifuged at 1500 rpm for 15 minutes, and the serum was transferred into an Eppendorf tube. It was kept frozen at -20°C until use.

Assessment of mouse Interleukin-12, IgG, and tumor necrosis alpha levels (TNFα) were conducted using ELISA KIT (KOMA BIOTECH INC, South Korea) based on manufacturing protocol.

Statistical analysis

The statistical analysis was done using SPSS software (version 22). A two-way analysis of variance (ANOVA) was used to analyze the data statistically. Duncan's test was chosen to determine the mean significant differences between treatments, and $p \leq 0.05$ considered significant (Snedecor and Cochran, 1980).

RESULTS

Delayed-type hypersensitivity

The skin test findings of mice post-immunization showed the means of skin thickness against both Ag were significantly high in the first (3.35 ± 0.18), second (2.40 ± 0.14), and third (3.62 ± 0.11) groups, compared to the fourth group (1.87 ± 0.08 , $p \leq 0.05$). However, these values decreased 48 hours (day 29) post-examination in all groups (Table 1).

Determination of immunized parameters

Table 2 shows the result of TNF α titer 30 days post-immunization. The findings indicated that TNF α titer significantly increased ($p < 0.05$) in the third (603.88 ± 21.6) and the first groups (598.05 ± 13.56), compared to the second (280.97 ± 23.83) and fourth groups (270.27 ± 140.65). The results of IL 12 titer also showed a significant ($p < 0.05$) difference in the third group (1382.27 ± 27.37), compared to other groups, and the control group was the lowest (252.12 ± 59.96). There was a significant difference in IgG concentration between groups first (6.80 ± 0.71) and third (6.79 ± 0.20) with other groups, and the control group was the lowest (2.54 ± 0.13).

Table 1. Skin thickness in immunized mice at days 28 and 29 of the experiment

Groups	day 28	day 29
Immunize with hydatid cyst sonicated protoscolex Ag	3.35 ± 0.18^{Aa}	1.92 ± 0.08^{Bb}
Immunize with <i>cysticercus tenuicollis</i> sonicated protoscolex Ag	2.40 ± 0.14^{Ba}	1.88 ± 0.07^{Bb}
Immunize with both Ag	3.62 ± 0.11^{Aa}	2.57 ± 0.13^{Ab}
Control	1.87 ± 0.08^{Ca}	1.41 ± 0.13^{Cb}

Values are expressed as mean \pm standard error. Different superscript letters (^{A,B,C}) means significant differences in a column ($p < 0.05$). Different superscript letters (^{a,b,c}) means significant differences in a row ($p < 0.05$). Ag: Antigen.

Table 2. Determination of immunized parameters (TNF, IL-12, and IgG) in mice within 30 days of the experiment

Groups	TNF α (pg/ml)	IL-12 (pg/ml)	IgG (ng/ml)
Immunize with hydatid cyst sonicated protoscolex Ag	598.05 ± 13.56^A	772.4 ± 210.28^B	6.80 ± 0.71^A
Immunize with <i>cysticercus tenuicollis</i> sonicated protoscolex Ag	280.97 ± 23.83^B	604.23 ± 32.83^B	4.01 ± 0.45^B
Immunize with both Ag	603.88 ± 21.6^A	1382.27 ± 27.37^A	6.79 ± 0.20^A
Control	270.27 ± 140.65^B	252.12 ± 59.96^C	2.54 ± 0.13^C

Values are expressed as mean \pm standard error. Different superscript letters (^{A,B,C}) means significant differences in a column ($p < 0.05$). Different superscript letters (^{a,b,c}) means significant differences in a row ($p < 0.05$). Ag: Antigen.

DISCUSSION

All immunized groups showed a significant increase in the thickness of mice footpads compared to the control group. The highest mean of thickness was on day 28 of the experiment. This result may be due to the higher sensitivity of antigens, epitopes, or peptides responsible for delayed-type hypersensitivity and effective immune responses in skin testing. Specific antigens could be easily engulfed, processed by antigen-presenting cells, then recognized by T-cells, and induce a strong hypersensitivity reaction. Therefore, the main cause of skin thickness is the aggregation of a large number of lymphocytes which may reach to hundred times more than in normal conditions, especially sensitized T lymphocyte that releases chemokine and attracts phagocytic cells. The early thickness of the skin may be due to the release of chemical mediator, which induces edema, congestion of blood vessels, and swelling at the inoculation site (Helou et al., 2021). According to Mahmoudzadeh-Niknam et al. (2007), the early delayed-type hypersensitivity (DTH) reaction to Ag in mice is caused by eosinophils, basophilic mast cells. As a result, the current findings might be the result of Ags-activated CD4+ and CD8+ T-cells, which play a role in triggering DTH. Following exposure to dendritic cells and Langerhans cells, the site moves from the epidermal layers to the lymph nodes, where they present antigens to T lymphocytes that secrete interferon via major histocompatibility entities (Hemmi et al., 2001).

An obvious marked cellular immune response with a significant increase in thickness was revealed in the mice group immunized with mixed Ag (*Fasciola hepatica* and *hydatid cyst* fluid antigen) before and after the challenge dose (Al-malki, 2012). A number of studies have also revealed new information on the effective response of the immune system showing that Antigen B (AgB, the major antigens of *Echinococcus granulosus*), and cytokine response in a study on rabbits immunized with *Echinococcus granulosus* AgB (Chemale et al., 2005; Nisreen and Wafaa 2017), which can skew type 1-type 2 cytokines toward a preferred Th2 polarization (Rigano et al., 2001; Al-malki, 2012).

A regulatory role for IL-12 in innate resistance in intermediate host infection has been suggested, and this was accompanied by a Th1 response (Al-malki, 2012). The role of AgB in the human inflammatory response is determined by its effect on polymorphonuclear cells (PMN) and its action in acquired immunity (Rigano et al., 2001). This result may be due to factors IL-2 and IL-12 dependent on the activation of T-cells (Spiering 2015; Condotta and Richer, 2017).

The AgB stimulates intricate immunological responses of IL 12. Among these are polarized Th2 reactions paired with Th1 reactions. It has been proposed that IL-12 regulates innate resistance in the infection of intermediate hosts, which is accompanied by a Th1 response (Al-malki, 2012). The primary immune system regulators are CD4+ cells, which come in two subtypes (Th1 and Th2) with distinct roles (Toes et al., 1997). Th1 activation can promote the cellular immune response by inducing CD8+ T-cell lymphocytes to release IL-2, IL-12, and IFN- γ (Gómez et al., 2021).

Al-Qaoud et al. (2008) revealed that immunization of mice with AgB led to elevated IgG1 and IgG2a. AgB induced more IL-4 when given intraperitoneally (IP). Moreover, polarization towards the Th2 response showed that subcutaneous AgB vaccination of Balb/c mice caused the formation of a significantly important amount of total IgG (especially IgG1) linked with other routes of immunization, such as intramuscular and intraperitoneal, which coincided with the Th2 response. The earliest quantifiable IgG response to AgB occurs 2 to 11 weeks after immunization in mice and sheep, and 4 weeks in vervet monkeys (Zhang et al., 2012). Early infections can cause a high cellular inflammatory response and pathologic changes. Elevated eosinophils, lymphocytes, and macrophages cause leukocytosis. Oncospheres produce necrosis, neutrophil, and macrophage invasion for 3-5 days (Finkelman et al., 1991). According to some studies, exposure to or immunization with *Taenia hydatigena* protects against *Taenia ovis* (Heath et al., 1979; El-Moghazy and Abdel-Rahman, 2012).

Taenia hydatigena infection has been shown to protect mice from *Taenia taeniaeformis*, *Taenia saginata* in calves, and *Fasciola hepatica* in sheep (Muku et al., 2020; Jansen et al., 2021). It should be highlighted that *Leishmania donovani* antigens are combined with *Cysticercus tenuicollis* fluid antigen as an adjuvant, which may help to explain why antibodies are produced. Adjuvants are chemicals added to vaccinations to boost their potency by changing the immune system's reaction to certain immune cells (NCI, 2010). The outcome of this study is the production of antibodies, which may be a result of the adjuvants used to boost a vaccine's effectiveness by modifying the immunological response to certain immune system cell types.

CONCLUSION

The two types and mixes of Ag have been demonstrated to generate humoral and cellular immunity in immunopathological investigations. The combined antigens (*Hydatid cyst* and *Cysticercus tenuicollis*) may be significantly more immunogenic than each Ag alone. The cross-reactivity and synergistic interactions might bring about the antigenic activity for the two investigated parasites. It is therefore suggested to apply different substances to vaccines to increase their effectiveness by altering the immunological response to certain immune system cells.

DECLARATIONS

Acknowledgments

A pathology laboratory/department of pathology, Veterinary medicine, Baghdad university, Iraq, funded this study, and the animals were acclimatized in the animal home of the University of Baghdad's College of Veterinary Medicine, Baghdad, Iraq.

Authors' contribution

The final manuscript draft was reviewed by all authors, who also gave their approval.

Competing interests

There is no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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