

A 53 KDa Glycan Antigen of Hydatid Cyst Wall May Involve in Evasion from Host Immune System

Abstract

Background: Recent studies have shown that similar host glycan antigens are expressed by helminths such as *Echinococcus granulosus* hydatid cysts to evade from host immune system. In this work to investigate these antigens further, immunological cross-reactivity between human sera and hydatid cyst wall antigens has been investigated. **Materials and Methods:** Hydatid cyst wall antigens were used in enzyme-linked immunosorbent assay and Western immunoblotting and probed with pooled sera of hydatidosis patients and healthy controls. Sodium metaperiodate treatment was used to investigate glycan antigens. **Results:** A band with molecular weight about 53 KDa reacted with both hydatid patients' sera and also normal human sera. It has been shown that this band was a glycan antigen. **Conclusions:** A 53 KDa glycan antigen of hydatid cyst wall that reacted with all human sera may have an important role for evasion from host immune system.

Keywords: Antigen, glycan, hydatid cyst, immune evasion

Introduction

Hydatid cyst in humans happens as a result of infection with the larval stage of the *Echinococcus granulosus*.^[1] In the life cycle of the parasite, canines play the role of definitive host, and usually, herbivores or omnivores are intermediate hosts.^[2] Hydatidosis is one of the most important zoonotic diseases in the world.^[3] The highest incidence of human and animal echinococcosis is seen in the temperate zones including South America, the Mediterranean region, Central Asia, China, Australia, and parts of Africa.^[4,5] The anatomical shape of hydatid cyst which is the larval stage of the parasite is a single fluid-filled cyst. The cyst wall is composed of two layers, inner layer or germinal layer and the outer layer or laminated layer.^[6,7] Laminated layer is directly contacts with host tissues, so it seems that plays an important role in host-parasite interactions.^[8] This layer is a large extracellular matrix that made of mucin with much of O-glycan glycoproteins.^[9,10] This carbohydrate-rich layer is known also as a "glycocalyx."^[11] It has been shown that glycan determinants of hydatid cyst can regulate the host immune response and therefore may affect the course of infection.^[12]

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Worms' ability to regulate the host immune system is a major cause of their long life, for example, protoscolex of *E. granulosus* can be alive up to many years in hydatid cyst of humans.^[13,14] It seems that the immune response regulation is beneficial for both the human host and the parasite.^[15,16] *E. granulosus* with two mechanisms can regulate the responses of the immune system: (1) inactive evasion (parasite remains safe from the harmful effects of the immune system with growing in a cyst) and (2) regulation of the immune system (the parasite has been actively interacted with the host immune system to reduce its effect).^[17,18] It has been shown that glycans produced by helminths are used to regulate the host immune system.^[18,19] As an example, both schistosome and nematode helminth glycans are largely responsible for altering the immune response toward Th2-type.^[20] Furthermore, it has been shown that most of the monoclonal antibodies generated against schistosome antigens recognized glycan epitopes, including Lewis^x-containing structures.^[21] Moreover, there are strong evidence indicting that glycans rather than protein antigens direct the immune response to helminths.^[22]

Recent studies have shown that similar host glycan antigens that are expressed by more

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helminths are identified by dendritic cells (DCs) through the lectin receptors.^[23,24] The helminths use “Glycan Gimmicky” to target DCs (one of the most important antigen-presenting cells) to regulate T-cell to anti-inflammatory responses. “Glycan Gimmicky” in helminths is said to use active strategies to target host glycan-binding proteins to survive for a long time.^[25,26] In the cell surface of DCs, there is one group of receptors, called C-type lectin receptors (CLRs) which identify pathogens’ motif glycan. Both the stimulation and modulation of the host immune system by helminths are determined by DC populations.^[27] DCs display on their surface different kinds of CLRs.^[28]

Regarding important role of laminated layer of hydatid cyst in host-parasite interaction, particularly in regulation of the host immune response^[29] in this study, immunological reaction of hydatid cyst wall antigens with different host sera has been investigated.

Materials and Methods

In this descriptive research, the study population consisted of sera of either patient with hydatidosis or normal human sera. To prepare the antigens, liver and lung hydatid cyst of sheep was collected from Khomeini Shahr slaughterhouse in Isfahan, Iran. At the first, the hydatid cyst fluid aspirated with a syringe and checked under the microscope for the presence of protoscolices. Following observation of the protoscolices, the cyst was included in the study. Afterward laminate and germinal layers were separated with a forceps, homogenized, sonicated in phosphate-buffered saline (PBS), centrifuged, and the supernatant stored at 20 as hydatid cyst wall antigen. Glycoprotein of this antigen was purified by chloroform-methanol extraction of glycolipid and glycoprotein. Normal sera ($n = 20$) and hydatidosis sera ($n = 20$) were collected from different hospitals in Isfahan Iran.

Enzyme-linked immunosorbent assay (ELISA) was performed as we published before.^[30] Briefly, ELISA plate was coated with crude cyst wall antigen or purified glycoproteins. The antigen-coated plates were washed three times in washing buffer and then incubated for 1 h with blocking buffer and again washed three times. Hydatidosis patients’ sera or normal human sera, diluted in incubation buffer, were added. Plates were washed, and a relevant enzyme conjugated was added. After incubation for 2 h, the substrate 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) was added, and the reaction was read in a spectrophotometer at 414 nm.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) was performed for antigens on 12% acrylamide gel under reducing and nonreducing conditions using Bio-Rad mini gel instrument. The gels were either stained with Coomassie blue or transferred to nitrocellulose papers (NCP) using Bio-Rad apparatus. The papers were then probed with different sera at appropriate

concentrations. Following washing with the buffer, the paper was probed with a secondary antibody against human IgG. Finally, appropriate substrate was added to develop reaction of sera with the bands of antigens. To inactivate glycan epitopes on NCP, the membranes were treated with 20 mM sodium periodate in 0.1 M sodium acetate and then incubated with 50 mM sodium borohydride in PBS prior probing with the antisera.

This study was approved by the Ethics Committee of Isfahan Medical University, and sample collection was obtained with a written informed consent of patients and healthy controls.

Results

Results of ELISA test showed that both sera of patients with hydatid cyst and also normal human sera reacted with hydatid cyst wall antigens, but mean optical density of patients’ sera was higher than that of normal sera [Table 1].

In Western immunoblotting, hydatid cyst wall runs under reducing and nonreducing conditions and probed with the sera of patients with hydatid cyst or normal human sera. Because in reducing condition, more bands were detectable, the experiments were carried out only in reducing condition. In reducing conditions, sera of patients with hydatid cyst and normal human sera reacted with some bands of the hydatid cyst wall. However, the reaction of sera of hydatid cyst patients was stronger than reaction of normal sera [Figure 1].

To check if reactivity of sera with hydatid cyst wall antigens related to carbohydrate epitopes, cyst wall antigen runs on SDS PAGE and transferred on NCP membrane. The paper was then cut into two halves. One half was treated with sodium metaperiodate and the other half left intact, and then, normal Western blotting was continued with hydatid cyst patients’ sera or normal human sera. Following sodium metaperiodate treatment, all bands including a 53 KDa band that has the highest reaction with our sera were removed [Figure 2].

Discussion

The results of this study demonstrated that both hydatidosis and normal human sera reacted with hydatid cyst wall

Table 1: Mean optical density of enzyme-linked immunosorbent assay plate coated with hydatid cyst wall or hydatid fluid antigens and probed with sera of patients with hydatid cyst or normal human sera

Coated antigens	Mean optical density results product from interaction with these sera		
	Hydatidosis patient sera	Healthy persons sera	Negative control
Cyst fluid	1.54	0.49	0.06
Cyst wall	0.66	0.50	0.06

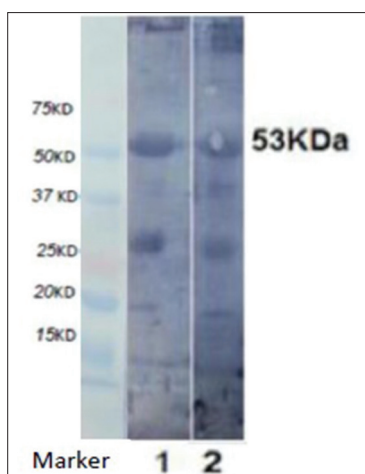


Figure 1: Western immunoblotting of hydatid cyst wall antigen under reducing condition probed with pool sera from patients with hydatid cyst (2) or normal human sera (1)

antigens and also it was shown that these antigens are glycosylated antigens. Hence, it can be concluded that hydatid cyst wall crude antigen is not a suitable antigen for immunodiagnosis of hydatid cyst.

In immunology of helminths' infection, it has been shown that the mechanisms of host immune system are not able to clear chronic infections,^[14,31] and carbohydrate epitopes of helminths may play a role in this regard. Considering the importance of glycan in helminths immunology,^[32] in this work, antibody response to hydatid cyst, especially glycosylated antigens, has been investigated.

It seems that laminated layer of *E. granulosus* plays an important role in host-parasite interactions.^[6,33] A number of antigens, including many glycoproteins, are produced by the metacestode of *E. granulosus* that can regulate host immune system. Furthermore, it has been shown that removal of the whole N-glycan of hydatid cyst causes a dramatic loss of immune reactivity.^[34]

Glycans are abundant on the surfaces of helminths and within their secreted antigens.^[35] Several studies show that recognition of pathogen-associated and host-like glycan antigens expressed by many helminths can interfere with the production of effective immune responses.^[23,36] The ability of helminths to induce chronic infection may be beneficial both for the hosts and the parasites. For instance, "Glycan Gimmicky" of helminths may be useful for host.^[25]

In our work, a 53 KDa glycan antigen of hydatid cyst wall that cross-reacted with normal human sera may have an important role for *E. granulosus* evasion from human immune system. Probably, the parasite produces this antigen to make the host produce antiglycan antibodies to block the site of effective antibodies such as IgE (blocking antibodies).^[37,38] In an investigation, sera of patients with hydatid cyst reacted with several bands of cyst wall in Western blotting.^[39] In another work, cross-reaction of sera of patients with breast cancer

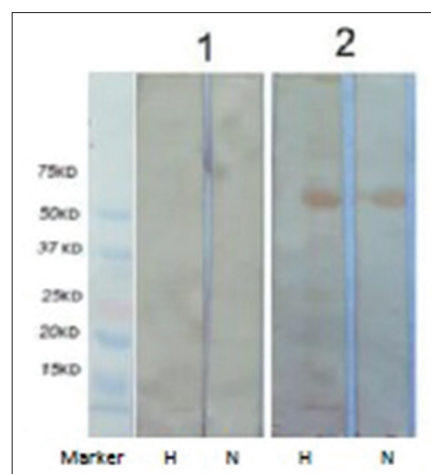


Figure 2: Western immunoblotting of hydatid cyst wall before (2) and after (1) sodium metaperiodate treatment probed with sera of patients with hydatid cyst (H) or normal human sera (N)

with a 27 KDa antigen of hydatid cyst wall has been shown. This band was sodium periodate-resistant antigen indicating that it was not a glycosylated antigen.^[40] However, none of the above-mentioned bands reacted with normal human sera. Hydatid cyst wall can allow antigens up to 150 KDa diffuse to host tissues.^[41] Hence, it is possible that 53 KDa antigen of hydatid cyst wall usually diffuses out to host tissue to regulate the immune system toward a nondestructive response. In agreement with our result, involvement of interleukin (IL10) and IL4 in evading of hydatid cyst from host immune response has been shown.^[42] In another work, possible role of the parasite antigen B in evasion from the host immune response has been suggested.^[43] Furthermore, complement evasion by *E. granulosus* hydatid cyst has been suggested.^[44]

Mechanisms of escaping parasite from immune system are sophisticated aspects of the host-parasite relationship.^[31] Hence, further investigation is recommended to find the exact role of hydatid cyst glycan antigens which cross-react with normal human serum and their involvement in evasion of the parasite from host immune response.

Conclusions

In this work it has been shown that a 53 KDa glycan antigen of hydatid cyst wall reacted with all human sera. So this antigen may have an important role for evasion from host immune system.

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Conflicts of interest

There are no conflicts of interest.

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