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Evaluation of rabbit antibody response against 8 and 16 kDa recombinant subunits of antigen B from *Echinococcus granulosus*

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ABSTRACT

Objective: To immunize rabbits with 12 and 16 kDa recombinant subunits of antigen B from *Echinococcus granulosus (E. granulosus)* and measuring polyclonal antibody and humoral immune response using ELISA and gel diffusion. **Methods:** Two mentioned antigens were cloned and expressed in expression vector and purified by affinity chromatography. Four young rabbits were selected and challenged intradermally with yielded recombinant antigens. Rabbits' sera were collected post infection and were tested using ELISA and gel diffusion for polyclonal antibody detection 10 days after last injection. **Results:** The specific antibody against the recombinant peptides was efficiently produced within 4 weeks post infection. **Conclusions:** Produced recombinants proteins could induce the immune response of the rabbits successfully. This process might improve the clarification of diagnosis and vaccination as regards hydatidosis.

1. Introduction

One of the most important and fatal helminthic diseases, caused by the larvae of the parasite not the adult itself, is cystic echinococcosis or hydatid disease. It is a larval stage of *Echinococcus granulosus* (*E. granulosus*), with canids as definite host and human, ruminants, and carnivorous as intermediate host[1]. The disease is common in most regions of the world especially in rural areas where usually ruminants are non-healthy slaughtered[2].

Hydatidosis encompasses a very long period of incubation and during this time normally has no obvious clinical manifestations. Thus, diagnosis of the disease needs extra devices than signs and symptoms solely. For more than 50 years, the various serologic tests have been applied for detection of antibodies against the disease including IgG, IgM, IgA and IgE[3]. Metacestod of *E. granulosus* is usually unaffected by immune response during the developing stage[4]. Specific antibodies against the *E. granulosus* antigens remain several years after treatment of the disease[5], therefore many of serological tests remain positive even after surgery or successful chemotherapy[4,6]. Metacestod of *E. granulosus* secret a polymeric lipoprotein known as Antigen B[7] and is encoded by a gene family[8]. Antigen B is composed of 8/12, 16, 24 kDa subunits[9]. Some researchers have already reported trials to evaluate different recombinant subunits of AgB in diagnosis of human hydatidosis with more or less successful results[9-12] but regardless all these challenges still there are dark spots on clarification of the proper understanding of immunity and diagnosis of the disease.

In the present study, 2 subunits of 12 and 16 kDa recombinant subunits of antigen B from *E. granulosus* were produced, purified, and then injected to rabbits. Afterwards, production of polyclonal antibody and inducing the humoral immune response were assayed by ELISA and gel diffusion.

2. Materials and methods

2.1. Production of recombinant antigens

We previously cloned 12 and 16 kDa subunits of antigen

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B from *E. granulosus* and expressed them in expression vector[10].

2.2. Induction of polyclonal antibody in rabbits

The method was conducted essentially based on a previous study[13] with some modifications. Four young white male rabbits, 2 year-old, each 2-2.5 kg, were enrolled in the study. Recombinant antigens were passed through 0.22 microns filter and emulsified with an equal volume (v/v) of Freund's complete adjuvant to enhance the response to the immunogen. Adult rabbits were injected intradermally, in different limbs. Before the injection of antigens, four challenged rabbits were bled. Injection procedure was performed as follows:

First injection: One mL antigen mixed with Freund's complete adjuvant was injected with a concentration of 500 μg antigen per mL; second injection: Two weeks later, one mg antigen mixed with incomplete adjuvant; third injection: Ten days after the second injection, one mg of antigen directly; and fourth injection: 10 d after the third injection, 500 μg of sterile antigen. Ten days after the last injection all rabbits were bled and sera were tested using ELISA and gel diffusion.

The study was approved by the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran.

2.3. Gel diffusion

Gel diffusion was conducted as stated earlier[14].

2.4. ELISA

ELISA was conducted as reported earlier[15] with some modifications. Briefly, the diluted r12 kDa and r16 kDa (2 μg/mL each) in the coating buffer were dispensed into the flat-bottomed wells of 96-well MaxiSorpTM plates (Nunc, Roskilde, Denmark) and left overnight at 4 ℃. After three washes, each well was filled with 1:200 dilution of each serum before the plates were incubated for 1 h at 37 ℃. After another three washes, the conjugate of goat antirabbit-IgG (c-chain) with horseradish peroxidase (Sigma Chemical Co., Poole, Dorset, United Kingdom) was added at a dilution of 1:10 000. After incubation, 0.4 mg/mL o-phenylenediamine solution (Sigma Chemical Co., Poole, Dorset, United Kingdom) was added. The absorbance of the contents of each well was then read at 492 nm, on an ELISA reader (Tecan, Männedorf, Switzerland), with a 620 nm reference filter.

3. Results

Figure 1 (A) shows the result of gel diffusion analysis of antibody prepared from a rabbit injected with 12 kDa recombinant subunit and (B) shows the result of same trial with 16 kDa recombinant subunit. Figures 2 and 3 show the result of ELISA test from rabbits immunized with r12 kDa and r16 kDa antigens, respectively. Optical density absorbances are mentioned bellow the figures.

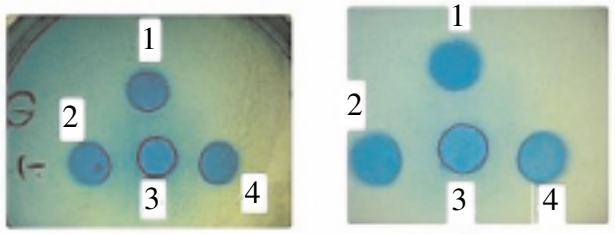

Figure 1. Gel diffusion results of rabbits' sera immunized with recombinant r12 kDa(A) and r16 kDa(B) antigens of *E. granulosus*.
1: Antigen; 2: Animal serum before injection; 3: Immunized rabbit's serum; 4: Bacterial lysate.

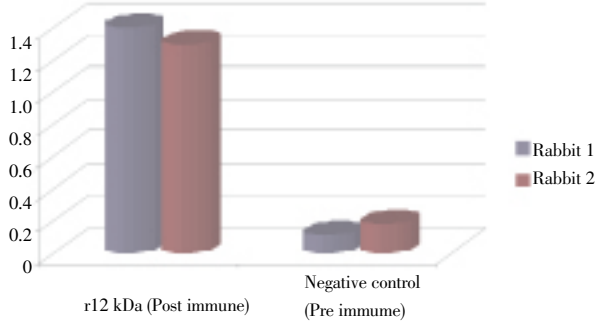

Figure 2. Antibody titer of two rabbits immunized with r12 kDa subunit of antigen B from *E. granulosus*.
Optical density absorbances of two rabbits before and after immunization were 0.11 and 0.18, as well as 1.25 and 1.37, respectively.

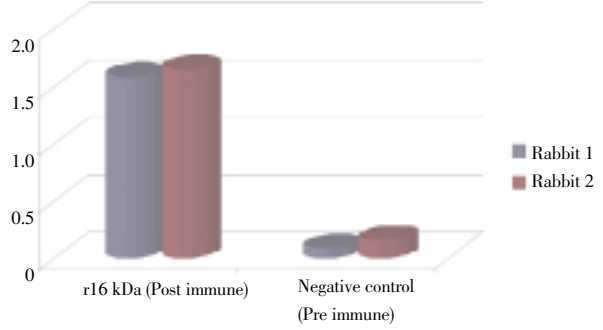

Figure 3. Antibody titer of two rabbits immunized with r16 kDa subunit of antigen B from *E. granulosus*.
Optical density absorbances of two rabbits before and after immunization were 0.09 and 0.16, as well as 1.56 and 1.47, respectively.

4. Discussion

In this study, we succeeded to induce and evaluate polyclonal antibodies in rabbits against 12 and 16 kDa recombinant subunits of antigen B from *E. granulosus*.

Polyclonal antibodies are important proteins utilized in a variety of laboratory techniques (*e.g.* vaccines, immunoblotting, immunoprecipatation, and diagnostic tests) in many fields of biomedical research. In the area of serodiagnosis and antibody production on human hydatidosis, antigen B and its subunits have been used by many researchers so far[9,11,16]. However, there are few studies to challenge recombinant AbB subunits on animals

to survey the production of polyclonal antibodies.

Our study showed that the expression and purification of recombinant antigens for the production of polyclonal antibodies in rabbit was practical. Hashemitabar *et al*., produced polyclonal antibody against hydatid fluid, protoscolex, and adult *E. granulosus* antigens in mice and concluded that the level of antibody against the adult worms was higher than those of hydatid cyst fluid and protoscolex[17]. Lithowlers *et al* used monoclonal antibody for determination of antigenic component of hydatid cyst especially antigens 5 and B[9]. Khaled *et al* applied generation of monoclonal antibody against B8/2 antigen of *E. granulosus* and appraised its specialty with crude hydatid cyst antigen and other parasites' antigens. They showed that this antigen had cross-reaction with *Schistosoma mansoni* antigen[18]. It has been shown that r*Eg*P29 elicits high titer of specific antibody in mice after immunization[19]. Immunogenicity of two antigens from *E. granulosus* including *Eg*A31 and *Eg*Trp in mice was surveyed by ELISA and these antigens induced high antibody titer[20]. However, all these trials have been conducted to ease the process of obtaining polyclonal or monoclonal antibodies to utilize them in diagnostic or vaccination techniques. We showed that r12 and r16 kDa antigens of *E. granulosus* are capable to induce immune system of a laboratory animal within 4 weeks post infection.

In conclusion, although, the level of antibody in rabbit immunized with r16 kDa was higher than that of r12 kDa but both antigens separately or in combination might be candidate for vaccination or diagnosing trials.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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