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Short Report

Protective Immunity in Mice with Whole Body of *Echinococcus granulosus*

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ABSTRACT

Despite the establishment of extensive and successful control programmes in some countries or regions, *Echinococcus granulosus* still has a very wide geographical distribution. Mature *E. granulosus* (n = 120) obtained from Parasitology Department, School of Veterinary Medicine, Ferdowsi University of Mashhad (Iran). Soluble protein of whole body of parasite was prepared by freeze-thawing in liquid nitrogen and at 42ºC for three times. The sample was homogenized in a blender, sonicated at 110 V, 170 W for 3 times for 15 s each and then centrifuged at 10,000 ×g for 15 min. Final yield was kept in -20ºC. Ten mice were randomly divided into 2 groups of 5. Each mouse in test group was vaccinated subcutaneously with 100 µg (100 µl) of whole body of *E. granulosus* plus 100 µl of Freund's complete adjuvant (FCA), respectively. Mice in control group were vaccinated with adjuvant in PBS. Second vaccination was conducted after four weeks with the same preparation except that FCA was replaced by Freund's incomplete adjuvant (FIA). Three weeks after the second vaccination, each mouse was challenged with 2000 protoscolices, intraperitoneally. Mouse autopsy was carried out eight months post challenge. Our results show that none of the vaccinated mice with the whole body of *E. granulosus* had cysts that indicate 100% protective immunity. *Iran. Biomed. J. 10 (1): 51-55, 2006*

Keywords: Hydatidosis, *Echinococcus granulosus*, Whole body, Mouse

INTRODUCTION

Cystic echinococcosis, caused by the larval stage of *Echinococcus granulosus*, is a global public health problem. Whilst in a few localities such as New Zealand, the parasite has been effectively controlled or even eradicated; in most endemic regions it remains a persistent problem [1]. In spite of successful control programmes in some countries or regions, the parasite still has a very wide geographical distribution. There is clear evidence for the emergence or re-emergence of human cystic echinococcosis in parts of China, central Asia, Eastern Europe, and Israel [2]. However, there is a clear need for new advances in the prevention of echinococcosis [3].

Because of economical and hygienic importance of hydatid cyst, investigation in preventive methods in enzootic areas is considerable. Variation of *E. granulosus* strains has a major role in control of hydatidosis because it may affect on transmission pattern. The detection of protective antigens in hydatid fluid, oncosphere and protoscolex for preparing a suitable vaccine is a major part of investigation. This vaccine will be valuable to control the transmission of this important human pathogen and it also has the potential to prevent hydatid disease directly through human vaccination. A protein (designated EM95), closely related to EG95, has been identified in *E. multilocularis* that can induce protection against challenge infection with *E. multilocularis* eggs in mice [4]. Potentially, vaccination could be useful if applied in human populations in endemic areas, especially in hyperendemic foci such as those described in parts of China [5]. For control of the *E. granulosus* infection in animal and human populations, clear

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strategies and efficient methods are available, but they are costly and have to be performed for years or decades. Persistence or re-emergence of this infection is well documented in some countries and is primarily caused by the lack or reduction of control measures due to economic or other reasons. Reinforcement of currently available control programmes could prevent re-emergence of the infection and result in effective control. However, easier and cheaper methods are required to improve control worldwide. A new potential in this respect is vaccination of livestock animals in order to interrupt the cycle on that level, in association with other measures [6].

This study deals with either the presence of hydatid cysts and also protective immunity after vaccination with whole body of *E. granulosus* antigen.

MATERIALS AND METHODS

Preparation of samples. Mature *E. granulosus* (n = 120), which were kept in 10% formalin for 5 months, obtained from Parasitology Department, School of Veterinary Medicine, Ferdowsi University of Mashhad (Iran). They were washed with Hank's solution for three times and after washing with PBS (pH 7.3), were kept in PBS at -20ºC. Soluble protein of mature *E. granulosus* was prepared by freeze-thawing in liquid nitrogen at 42ºC for three times. Then, the sample was homogenized in a blender. The sample was sonicated at 110 V, 170 W (ultrasonic disintegrator, Hielscher, Germany) for three times each 15 s on ice and then centrifuged at 10,000 ×g for 15 min. Finally, the sample was filtrated with a 0.22-µm filter. Final yield was measured by Bradford method and kept in -20ºC until use.

Vaccination and challenge. Ten six-week-old BALB/c mice were divided into 2 groups of 5. Each mouse in test group was vaccinated subcutaneously with 100 µg of whole body of *E. granulosus* protein dissolved in 100 µl of PBS plus 100 µl of Freund's complete adjuvant (FCA). Mice in control group were vaccinated with 100 µl of adjuvant plus 100 µl of PBS. Second vaccination was conducted after four weeks with the same preparation except that FCA was replaced by Freund's incomplete adjuvant. Three weeks after the second vaccination, each mouse was challenged with 2,000 protoscolices intraperitoneally. All mice were killed 240 days after challenge and the internal organs were examined for hydatid cysts.

Histopathological examination. Tissue samples were taken from liver of control and immunized mice. Samples were fixed in buffer formalin 10% by routine method and then histopathologic section was prepared. Samples were stained by hematoxylin and eosin and finally were observed by a microscope.

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RESULTS

Our results showed that in all of the control mice there were a lot of cysts in internal organs after 8 months. The average size of hydatid cysts in control groups was 7.5 mm. The number of hydatid cysts in each of control mice was more than 50 (Fig. 1). In control mice, the number of hydatid cysts in liver and lung was 75%, in spleen 10%, in mesenteric 5%, in stomach 1%, in kidney 2%, in heart 1%, in diaphragm 2% and in subcutaneous 4%, respectively. In contrast, none of the vaccinated mice had viable hydatid cysts (Fig. 2). This result indicated that the protective immunity in our study with whole body of *E. granulosus* was 100%.

In histopathological exam, no microscopic cyst was observed in control mice. In contrast, lots of microscopic cysts were observed individually or multiple in immunized mice (Fig. 3).

In control mice, histopathological examination of the cyst revealed typical double layered wall and in some cases with clear fluid inside. Some samples revealed a precystic structure, which consisted of connective tissue and scattered hyaline cells showing a necrotic basophilic structure that

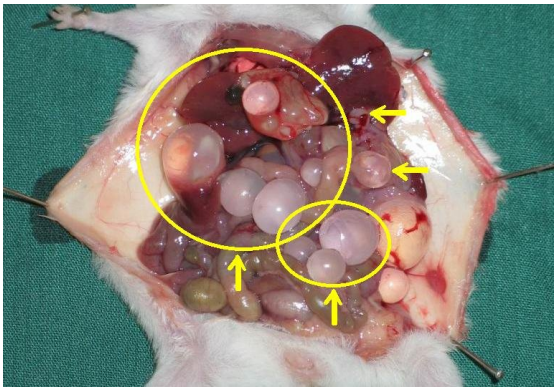

Fig. 1. Hydatid cysts in internal organs of mouse in control groups. The arrows indicate hydatid cysts in control mice.

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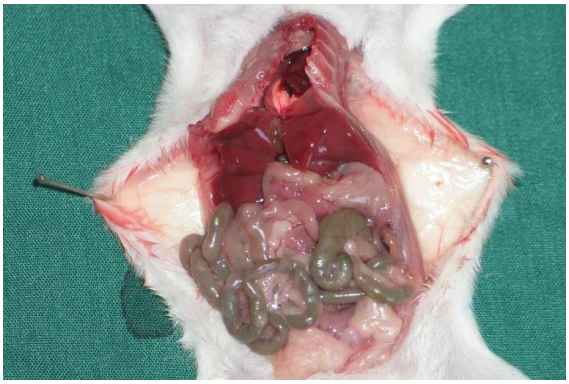

Fig. 2. Mice vaccinated with whole body of *E. granulosus* antigen. None of the vaccinated mice had viable hydatid cyst.

resembled a cubicula membrane. In some samples, beside infiltration of mononuclear and eosinophil, hyperplasia of bile ducts were observed in portal area (Fig. 4).

DISCUSSION

In our study, we found that the protective immunity in vaccinated mice was 100%. A BALB/c mouse model was used to determine which stage of the *E. granulosus* life cycle possessed the most potent protective antigens. Mice were immunized with crude extracts of protoscolices, brood capsules, cyst fluid, adult worm tissue, eggs or oncospheres and then challenged intraperitoneally with 600 activated oncospheres [7].

Osbon and Heath [8] used methods developed successfully for production of host-protective antigens from *Taenia* species to prepare excretory/secretory products from *in vitro* culture supernatants in which activated *E. granulosus* oncospheres had been maintained. Lambs that received two immunizations with these oncospheral antigens were protected against a subsequent oral challenge infection with *E. granulosus* eggs. While all control sheep developed numerous hydatid cysts as a result of the challenge infection, seven of eight vaccinated sheep were completely protected by the immunization. Dempster *et al*. [9] found that sheep which had been immunized with either sonicated *E. granulosus* oncospheres or the 100,000×*g* supernatant of SDS-solubilized oncospheres were moderately immune to an oral challenge infection with *E. granulosus* eggs. A higher level of immunity was achieved in sheep against *E. granulosus* by Heath and Lawrence [10] using oncosphere antigen, possibly because they used substantially greater quantities of antigens than did Dempster *et al.* [9]. Several antigen-expressing clones were selected and subcloned into a pGEX vector and then the GST fusion proteins tested in vaccine trials in sheep. The product of one clone (EG95) induced the highest level of protection in vaccinated sheep [11]. The results of the three vaccine trials in Australia and Argentina found that 86% of vaccinated sheep were free of any viable hydatid cysts when examined approximately one year after challenge infection [12]. Subsequent investigations have clearly identified only a single oncosphere antigen as being associated with EG95 and these studies have estimated the native antigen as being approximately 23 kDa [13, 14].

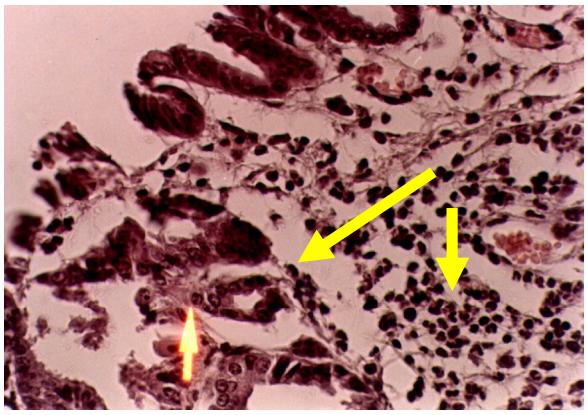

Fig. 4. The long arrow indicates hyperplasia of bile ducts and the short arrow indicates infiltration of mononuclear cells and eosinophils in portal area, respectively.

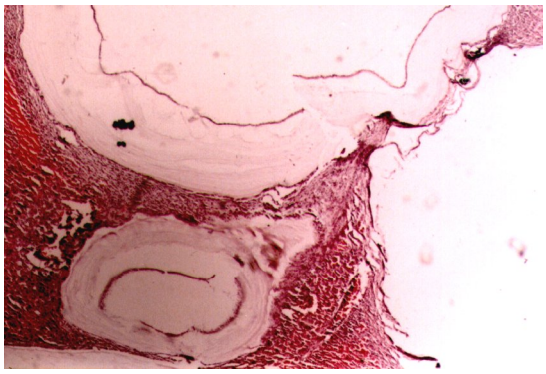

Fig. 3. Microscopic cysts in immunized mice and tissue reactions around cysts. The arrows indicate microscopic cysts.

A mouse model has been developed to evaluate potential protective antigens which could render intermediate hosts resistant to a challenge infection with *Echinococcus granulosus* eggs [15]. A large-scale safety and efficacy trial involving 50,000 and 100,000 lambs in Qinghai and Xinjiang Provinces

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of China has taken place by using EG65 antigen. Results have confirmed the safety and efficacy. The vaccine proved to be safe and well-tolerated, and after two injections protected kids against an artificial challenge infection by 100% at one month and 83% at six months. Lambs were protected by 91% at one month, 84% at six months and 97% at 12 months [16]. Highly effective recombinant vaccines have been developed against the helminth parasites *Taenia ovis*, *Taenia saginata* and *E. granulosus*. These vaccines indicate that it is possible to achieve a reliable, high level of protection against a complex metazoan parasite using defined recombinant antigens [17].

The EG95-based vaccine protects sheep from infection with the dog tapeworm *E. granulosus*. The EG95 encoding gene is a member of a multigene family, several members of which are expressed in the oncosphere, believed to be the target of immunity induced by the vaccine [18].

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