Trials to Induce Protective Immunity in Mice and Sheep by Application of Protoscolex and Hydatid Fluid Antigen or Whole Body Antigen of Echinococcus granulosus

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Summary

In the current study, soluble proteins prepared from 200 mature Echinococcus granulosus and protoscoleces of sheep hydatid cysts were applied to immunize sheep and mice respectively. The samples were mechanically homogenized in a blender, sonicated and the final yield was maintained at −20°C until analysis. Hydatid fluid was isolated from liver or lung of sheep under sterile conditions. In the first experiment, 15 mice were randomly allocated to three groups of five mice each. Each mouse in groups 1 and 2 was immunized with 100 μg of hydatid fluid and protoscoleces proteins in 100 μl of phosphate-buffered saline (PBS) and emulsified with an equal volume of Freund’s complete adjuvant (FCA) respectively. The mice of group 3 were immunized with adjuvant in PBS. The mice were boosted 4 weeks after the first vaccination with the same preparation except that FCA was replaced by Freund’s incomplete adjuvant (FIA). In the second experiment, eight male or female lambs 4–6 months of age, were allocated to two groups of four lambs each. Each lamb in the test group was vaccinated subcutaneously in the neck with a 2-ml dose of vaccine (1 mg of whole body protein of E. granulosus dissolved in 1 ml of PBS plus 1 ml of FCA). Control lambs were vaccinated with adjuvant in PBS. Lambs were boosted the same way as in the first experiment. Three weeks after the second vaccination, each mouse and lamb received a challenge infection with 2000 protoscoleces intraperitoneally and each lamb additionally received 10 gravid E. granulosus. All mice and sheep were killed after 7 months and examined for hydatid cysts. In these studies, protective immunity was induced in mice with protoscolex protein and with hydatid fluid, and in sheep with whole-body homogenate of E. granulosus and the levels of protection afforded were found to be 72.1, 82.6 and 90.9% respectively.

Introduction

Echinococcus granulosus has a worldwide geographical range and occurs in all continents and in circumpolar, temperate, subtropical and tropical zones. The highest prevalence of the parasite is found in parts of Eurasia, Africa, Australia and South America (Eckert et al., 2000). Although many species of domestic livestock and herbivorous wildlife species are potential hosts of E. granulosus, sheep play a major role in transmission of the parasite globally (Lightowlers et al., 1999). Despite ongoing control efforts, few countries have been able to substantially reduce or eradicate alveolar or cystic echinococcosis (Conchedda et al., 2002). The intermediate hosts of E. granulosus are long-lived and infection by eggs provokes a high degree of protective immunity, a characteristic that has been used for the development of a highly effective vaccine. Any success in recognizing the immunologic reactions of intermediate hosts and finally in producing an effective vaccine could lead to a decline in human infections and also in economic loss of animals. A vaccine to protect grazing animals against infection is an additional control method that focuses on grazing animals instead of the dog. Most grazing animals are already vaccinated against viral or bacterial diseases, and so a vaccine against a parasitic disease can fit into normal farm practice (Heath et al., 2003). Ongoing investigations will shed light on the biological roles played by the proteins within the parasites and the mechanism by which they make the parasites vulnerable to vaccine-induced immune responses (Lightowlers et al., 2003). The extraordinary effectiveness of the hydatid vaccine in the parasite’s natural animal hosts singles this vaccine out as having perhaps the greatest potential for development of the first effective human vaccine against a parasitic disease (Lightowlers, 2002). In the current study, the presence of hydatid cysts and also protective immunity after vaccination of sheep with whole body of E. granulosus and mouse with protoscolex and hydatid fluid antigens were investigated.

Materials and Methods

Preparation of antigens

Twenty cystic livers or lungs of sheep were collected from the abattoir of Mashhad. Hydatid fluid was isolated from livers or lungs in sterile conditions and centrifuged at 5000 g for 30 min (4°C) to remove protoscoleces and stored at −20°C. Two hundred mature E. granulosus were obtained from the Parasitology Department, School of Veterinary Medicine, Ferdowsi University of Mashhad, and isolated protoscoleces were washed with Hank’s solution three times. Soluble protein of mature E. granulosus and protoscoleces was prepared by three freeze–thawing cycles in liquid nitrogen and 42°C. Then the samples were homogenized in a blender. The samples were sonicated at 110 V, 170 W (Ultrasonic Disintegrator, Hilscher, Germany) for 3 × 15 s on ice and then centrifuged for 15 min at 10 000 g. Finally, the sample was sieved through a 0.22-μm filter and maintained at −20°C until analysis.

Protein concentration and dialysis

Protein concentration of hydatid fluid and homogenates of protoscoleces and mature E. granulosus were determined as described by Bradford (1976). In the case of low concentration,