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Immunisation of lambs against *Echinococcus granulosus* using antigens obtained by incubation of oncospheres in vitro

P. J. OSBORN, D. D. HEATH

Wallaceville Animal Research Centre, Research Division, Ministry of Agriculture and Fisheries, Private Bag, Upper Hutt, New Zealand

Activated oncospheres of *Echinococcus granulosus* were incubated in vitro for 14 days. The used culture medium was concentrated, emulsified with Freund's incomplete adjuvant and injected into lambs. These lambs subsequently displayed a very high level of resistance to an oral challenge with eggs of *E granulosus*. Serum collected at the time of challenge from vaccinated animals was extremely effective in killing oncospheres in vitro.

IMMUNITY of sheep to infection with *Echinococcus granulosus* has been considered to operate against two phases of the parasite's life cycle: pre- and post-encystment (Gemmell and Soulsby 1968). Immunity induced by antigens derived from cysts of *E granulosus* has been shown to act against the post-encystment phase, resulting in a reduction in growth of the cyst and calcification of parasite tissue. However, no immunity to the pre-encystment phase was stimulated and the same number of cysts established as in the controls (Turner et al 1937).

In contrast, live organisms stimulate a strong immunity to the pre-encystment phase. Sweatman et al (1963) showed that approximately 70 per cent of a challenge infection was prevented from establishing following a prior oral infection and Gemmell (1966) showed that parenterally administered eggs or oncospheres growing at the site of injection stimulated a high level of immunity to both the pre- and post-encystment phases. More recently, Heath et al (1981) showed that it was the early stages of the development of *E granulosus* that induced pre-encystment immunity.

Antigens produced during the early stages of larval development in vitro of many other taeniid cestodes have been used to immunise their intermediate hosts. Lambs have been immunised against *Taenia ovis* (Rickard and Bell 1971) and *T hydatigena* (Onawunmi and Coles 1980), calves against *T saginata* (Rickard and Adolph 1976), rabbits against *T pisiformis* (Heath 1976) and rats against *T taeniaeformis* (Rajasekariah et al 1980). The purpose of the present experiment was to determine whether antigens produced by *E granulosus* oncospheres in vitro were able to induce resistance in sheep to infection with this parasite.

Materials and methods

Antigen preparation

Eggs of *E granulosus* were obtained, washed, surface sterilised, hatched and activated as described by Heath and Lawrence (1981). The technique of producing antigens was based on the method used for *T ovis* by Rickard and Bell (1971). Four hundred thousand activated oncospheres of *E granulosus* were incubated for 14 days in 40 ml of medium NCTC 135 (Gibco, New York) containing 260 mg/litre L-cysteine and 20 per cent serum from a lamb not infected with larval cestodes. The medium was changed at two-day intervals, used media being filtered (0·2 μm membrane) and stored aseptically at 0°C. At the end of the culture period, the used medium was pooled, concentrated to a volume of 20 ml by dialysis against polyethylene glycol 6000 and stored at —20°C.

Animals and experimental procedures

Sixteen eight-month-old Romney lambs, reared on 'taeniid-free' pasture were randomly divided into two groups of eight. Lambs in the first group were injected intramuscularly in the thigh with the antigen preparation emulsified with an equal volume of Freund's incomplete adjuvant such that each animal received the secretions of 20,000 activated oncospheres. Six weeks later this immunisation procedure was repeated subcutaneously. Control lambs received no injection since previous studies with *T ovis* have shown no difference in susceptibility to a challenge infection between sham-vaccinated and untreated control animals (Osborn et al 1982).

Two weeks after the second injection, lambs of both groups were challenged orally with 1300 eggs of *E granulosus*. At the time of challenge, all lambs were bled to provide serum for examination of its ability to kill developing *E granulosus* oncospheres in vitro. The technique used was that of Heath and Lawrence (1981).

At necropsy, six months after challenge, the livers and lungs of all animals were finely sliced and the slices palpated to determine the number and viability of *E granulosus* cysts present. Samples of cysts were fixed in 10 per cent neutral buffered formalin, dehydrated and embedded in paraffin, cut at 4 μm and stained with haematoxylin and eosin.

Results

Only one vaccinated lamb had cysts present at necropsy and these were all located in the lungs (Table 1). The cysts of the control animals were generally equally distributed between liver and lungs. Cysts in the liver had a mean diameter of 2 mm (range 1 to 5 mm). Cysts in the lungs were larger, with a mean diameter of 4 mm (range 2 to 6 mm). Histologically there was no difference between cysts from control animals and those in the vaccinated animal and all appeared viable. The ability of the sera from vaccinated

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TABLE 1: Number of *E granulosus* cysts present in vaccinated and control lambs following oral infection with 1300 eggs of *E granulosus*

Group	Number of cysts in individual lambs							
Vaccinated	0	0	0	0	0	0	0	8
Control	134	40	281	226	159	200	81	171

animals to kill oncospheres was very significantly greater ($P<0{\cdot}001$, Mann Whitney U test) than that of the control animals (Table 2). Among the vaccinated animals, serum from the one lamb that did have cysts had the lowest oncosphere-killing ability in vitro.

Discussion

This experiment has demonstrated that antigens derived from the in vitro incubation of activated oncospheres of *E granulosus* can stimulate a very high level of immunity to a subsequent oral challenge with eggs of this parasite. This immunity appears to be directed only against the pre-encystment phase of the parasite's development, since no cysts were found in seven of the vaccinated lambs and the few cysts recovered from the one vaccinated animal appeared viable on histological examination.

It now seems likely that immunity to *E granulosus* in sheep is directed against the very early stages of the pre-encystment phase of the parasite's development. First, oncospheres release their protection-inducing antigens within 14 days and, secondly, the relationship between the rapid (24 hours) destruction of oncospheres in the serum of vaccinated animals and their subsequent resistance to a challenge infection indicates that it may be this stage that is attacked in vivo. The situation thus appears very similar to the immunity stimulated by the larval stages of many other taeniids, as mentioned previously, and also supports the findings of Xylinas et al (1976) who were able to immunise mice against an intraperitoneal challenge with *E granulosus* by repeated injection of an antigen prepared from *E granulosus* eggs.

The results of this experiment have shown for the first time that secreted antigens of *E granulosus* can be used to immunise sheep against infection. This crude vaccine now needs to be further developed, especially with regard to determining the most efficient dose rate and duration of immunity, so that it can provide a practical control measure against *E granulosus*.

TABLE 2: Percentage of *E granulosus* oncospheres killed after 24 hours in vitro in the serum of vaccinated and control sheep collected at the time of challenge

Group	% oncospheres killed by serum from individual lambs							
Vaccinated	97	98	97	98	89	98	97	73
Control	16	13	21	14	13	27	13	11

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