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Comparison of the antigenicity of protoscoleces and microvesicles of *Echinococcus multilocularis* prepared from rats

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Abstract

Rats are known to be relatively resistant to infection with *Echinococcus multilocularis*. However, when rats are inoculated with the parasite tissues, *E. multilocularis* proliferates slowly at first but after 6 months the cysts increase in size considerably and contain large numbers of protoscoleces. As rats survive for 18 months or longer, approximately 100 ml of packed protoscoleces can be produced from each rat. A comparison of the antigenicity of the protoscoleces and microvesicles by immunoblot methods showed that both Em18 and Em16 are shared components between both protoscoleces and microvesicles, although the latter have some additional antigenic components. In antigens prepared from protoscoleces, the banding patterns around Em18 were much simpler than those from microvesicles. Therefore, for serodiagnosis of *E. multilocularis*, antigens should be carefully prepared from protoscoleces rather than microvesicles from the rat.

Introduction

Alveolar echinococcosis (AE) has spread to some parts of the Northern Hemisphere (Craig *et al.*, 1992, 1996; Schantz *et al.*, 1995). As AE is one of the most lethal of zoonotic parasitic diseases, an early detection in patients is urgently required. We have reported that both Em18-immunoblot (Ito *et al.*, 1993, 1999) and Em2^{plus}-ELISA (Gottstein *et al.*, 1993) are highly reliable for detecting AE (Ma *et al.*, 1997) although Em18 appears to be more specific (Ito *et al.*, 1998). However, there is some controversy over the specificity of Em18 (Nirmalan & Craig, 1997). In the present study, we compared the antigenicity of the protoscolex and microvesicle by immunoblot analysis with the view to differentiate Em18 more clearly. For this work, we used commercially available Wistar rats for secondary experimental infections of *Echinococcus multilocularis*. Although the rat has been shown to be resistant to *E. multilocularis* (Ito *et al.*, 1996), we emphasize that rats are highly susceptible at least for secondary infections and therefore the rat might be an appropriate laboratory animal model for preparation of large amounts of protoscoleces.

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Materials and methods

Specific pathogen free, 5 to 6-week-old females of both closed colonies of Wistar rats and CD-1 (ICR) mice, purchased from CLEA Japan (Tokyo) and Charles River Japan (Tokyo), respectively, were used for experimental secondary infections with *E. multilocularis*. Protoscolex rich suspension with approximately 1×10^3 protoscoleces

Table 1. Recovery of larval tissues and protoscoleces of *Echinococcus multilocularis* from secondary infections in female Wistar rats and ICR mice.

Animals	Months after inoculation	Weight of larval tissues (g)	Volume of packed protoscoleces (ml)
Rat-1	13	214.9	33.5
Rat-2	15	256.3	35.0
Rat-3	15	384.6	65.0
Rat-4	15	185.0	28.5
Rat-5	15	325.2	53.6
Rat-6	18	437.5	83.4
Rat-7	18	546.2	96.8
Rat-8	18	467.2	N.T.
Mouse-1	8	10.3	1.6
Mouse-2	8	8.5	0.8
Mouse-3	8	7.2	0.7
Mouse-4	10	10.5	2.0
Mouse-5	10	14.2	2.5
Mouse-6	10	10.3	1.6

14 of 20 mice died within 6 months.
N.T., not tested.

ml^{-1} sterile PBS was inoculated into each host intraperitoneally. Following experimental infection, animals were killed every two or three months for up to 18 months. According to the density gradient method of Kanazawa *et al.* (1995), tissues of *E. multilocularis* recovered from rats were utilized for the preparation of protoscoleces and especially for microvesicles. The preparation of protoscoleces was carried out in a round-bottomed glass dish, a gentle circulation of which resulted in an accumulation of protoscoleces in the centre of it. Both protoscoleces and microvesicles were checked for purity under a microscope and subsequently homogenized (Econo-grind homogenizer, Radnotic Glass Technology, USA). All procedures for SDS–PAGE and immunoblot were carried out as described by Ito *et al.* (1993). Commercially available pre-cast 4–20% gradient gels (No. 01–022, SDS–PAGE Mini; TEFCO, Tokyo, Japan) were used for SDS–PAGE (Ito *et al.*, 1993).

Results and Discussion

Preliminary observations from more than 20 Wistar rats indicated large volumes of *E. multilocularis* appearing after at least 8 or 10 months following experimental inoculation with the parasite tissue homogenates. Table 1 provides a summary of the biomass of both larval tissues and protoscoleces of *E. multilocularis* recovered from rats and mice. In rats, the larval tissues developed up to approximately 550 g in weight and over 90 ml of packed protoscoleces were readily prepared from such tissues, whereas the majority of mice had died within 6 months and the maximum weight of the larval tissues was only 14 mg and the maximum volume of packed protoscoleces was less than 3 ml.

Basically, it was not difficult for us to prepare both microvesicles and protoscoleces by the density gradient method (Kanazawa *et al.*, 1995) but the preparation of protoscoleces was much easier, in view of the large numbers present in infected rats. The comparative immunoblots of both microvesicles and protoscoleces indicates some sharing of antigenic components, although the Em18 band appeared to be much richer in protoscolex antigens and without bogus bands around it (fig. 1).

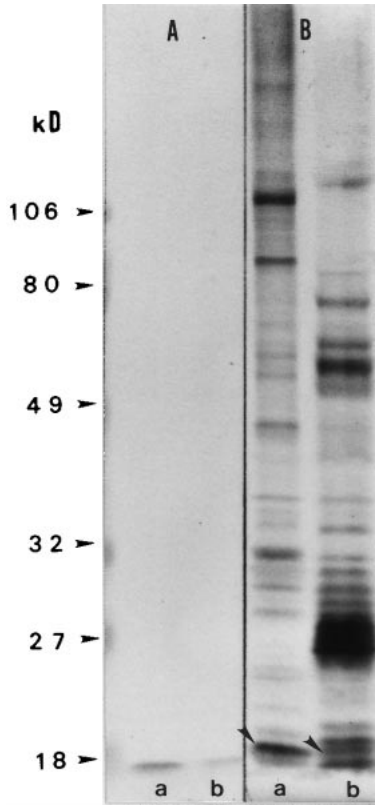

Fig. 1. Immunoblots of protoscoleces (lane a) and microvesicles (lane b) of *Echinococcus multilocularis*. Panel A was treated with monoclonal antibody against Em16 and Panel B with serum from a patient with alveolar echinococcosis (Em18 arrowed).

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This is the first report to demonstrate that the rat, apart from being a good laboratory model for experimental secondary infections of *E. multilocularis*, provides a large source of protoscoleces, especially after one year of infection. The mass of parasite tissues recovered from each rat is at least 20 times more than that from a mouse (table 1). However, mice and jirds harbouring secondary infections of echinococcosis die within 6 months and appear to be too susceptible for the parasite to proliferate large cysts, either with or without protoscoleces (Nakaya *et al.*, 1997). In contrast, in the present study, the Wistar strain of rat, which survives over a longer period, appears to be a better laboratory model for the preparation of large amount of protoscoleces at least.

When the antigenicity of microvesicles and protoscoleces was compared by immunoblot analysis, it became evident that: (i) microvesicles had some unique components which differed from those of the protoscoleces; (ii) the Em18 band, a most reliable serological marker for the differentiation of AE from other diseases (Ito *et al.*, 1999), was much enriched in protoscoleces; and (iii) there were many bogus bands around the Em18 band in crude antigens of microvesicles. When the purity of protoscoleces was not carefully checked, Em18 and Em16 bands were difficult to identify, but this can be overcome by purifying Em18 enriched fraction from crude antigens by isoelectric focusing (Ito *et al.*, 1999). Nirmalan & Craig (1997) demonstrated a broad band around Em18 using crude antigens from protoscolex enriched parasite tissues. However, as shown in fig. 1, if the parasite tissue contained microvesicles, the banding patterns become confused and Em18 or Em16 are sometimes impossible to identify, especially without reference to a monoclonal antibody against Em16. Therefore, the optimum conditions for identification of Em18 from crude antigens of protoscoleces are to use: (i) crude antigens from protoscoleces without contamination of microvesicles; (ii) 4–20% gradient gels for SDS–PAGE; and (iii) monoclonal antibody against Em16. Even if the monoclonal antibody cannot be used against Em16, it is not difficult to identify Em18 under these conditions. As Em18 enriched fractions can be purified from the crude antigens (Ito *et al.*, 1999), this makes serodiagnosis of AE much easier. However, for routine serology, crude antigens of protoscoleces are sufficient to identify or differentiate AE from the majority of other diseases (Ito *et al.*, 1998, 1999).

Although protoscoleces are not always well proliferated in AE patients, Em18 prepared from protoscoleces is a good marker for identification of AE and approximately 90% of AE cases are easily detectable by an Em18-immunoblot (Craig *et al.*, 2000). This is due to the fact that Em18 is shared between protoscoleces and microvesicles (fig. 1). Furthermore, there are some predominant components unique to microvesicles (fig. 1) and these components might be good candidates for the detection of early stages of AE.

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