IMMUNIZATION OF RODENTS AGAINST *HYMENOLEPIS* INFECTIONS USING NON-VIABLE HOMOLOGOUS ONCOSPHERES

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Immunity to Taiwan *Taenia* infection in pigs can be stimulated using homologous or heterologous non-viable *Taenia* oncospheres. This study was designed to determine whether homologous non-viable oncospheres could stimulate immunity to *Hymenolepis* infection in rodents. Hatched oncospheres were prepared from eggs of *Hymenolepis diminuta*, *Hymenolepis nana*, and *Hymenolepis microstoma* and kept at –70°C for more than 1 month. A mixture of 500 non-viable oncospheres of each tapeworm and complete Freund's adjuvant was injected subcutaneously in four groups of Sprague-Dawley rats or ICR mice one to four times at an interval of 1 week; controls were not immunized. After immunization, each rodent was orally inoculated with three fresh active cysticercoids of *H. diminuta* or *H. microstoma* or 500 fresh eggs of *H. nana*. The animals were then necropsied for adult tapeworms. No rats or mice immunized with non-viable oncospheres of *H. diminuta* or *H. nana* were infected by the challenge inoculation. However, 28 of 34 mice immunized with non-viable *H. microstoma* oncospheres were infected after inoculation with cysticercoids. This study demonstrated complete protection against infection by homologous parasites in rats or mice immunized with non-viable oncospheres of *H. diminuta* and *H. nana*, respectively. Repeated immunization may not be required if resistance is stimulated in rodent hosts.

Key Words: *Hymenolepis diminuta*, *Hymenolepis nana*, *Hymenolepis microstoma*, immunization, oncospheres
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A single oncosphere of *Hymenolepis nana* in the intestinal villus can elicit complete protection against subsequent egg challenge in mice within 2 days [1–3]. Moreover, mice initially infected with *H. nana* eggs become completely resistant to challenge with mouse-derived cysticercoids after more than 10 days [4]. Although no worm expulsions or worm senescence of *Hymenolepis diminuta* occurs throughout the life span of the rat host when about 10 or fewer worms are initially established [5–7], immunologically mediated rejection of this parasite by the rat have been reported [8]. Mice infected with *Hymenolepis microstoma* have also been reported to be resistant to reinfection [9]. These findings suggest that mice and rats could be immunized using vaccines against *Hymenolepis* species. Recently, we succeeded in showing cross-protection against *Taenia taeniaformis* in rats immunized with non-viable oncospheres of Asian *Taenia* or *Taenia saginata* [10]. Immunity to Taiwan *Taenia* infection in pigs can also be stimulated using homologous or heterologous non-viable oncospheres of *Taenia* species (Fan et al, unpublished data). It is worth investigating whether homologous non-viable oncospheres can stimulate immunity to *Hymenolepis* infection in rodents.

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Materials and Methods

Preparation of vaccines

Eggs of *H. diminuta*, *H. nana*, and *H. microstoma* were prepared from the gravid proglottids of tapeworms established in mice or rats in our laboratory [11]. The oncospheres were hatched and activated *in vitro* using the method described by Stevenson [12]. These hatched oncospheres were then suspended in Eppendorf tubes containing sterile phosphate-buffered saline (PBS) and immediately stored at –70°C for more than 1 month before use.

Immunization

For experiments with *H. diminuta*, 15 male Sprague-Dawley rats (150 g) were divided into five groups, four experimental groups and a control group. A mixture of 500 non-viable *H. diminuta* oncospheres (0.2 mL) and 0.2 mL complete Freund's adjuvant (CFA) was injected subcutaneously, once in the first group, twice in the second group, three times in the third group, and four times in the fourth group. The interval between successive immunizations was 1 week. In the control group, rats were injected subcutaneously with a mixture of PBS (0.2 mL) and CFA (0.2 mL).

For experiments with *H. nana* and *H. microstoma*, 25 male ICR mice (25 g) were used for each species, divided into five groups. A mixture of 500 non-viable oncospheres (0.2 mL) and 0.2 mL CFA was injected subcutaneously, once in the first group, twice in the second group, three times in the third group, and four times in the fourth group. The interval between successive immunizations was 1 week. In the fifth, control, group, mice were injected subcutaneously with a mixture of PBS (0.2 mL) and CFA (0.2 mL).

Experimental infection, sacrifice, and examination

Rats were challenged with *H. diminuta* by oral inoculation with three fresh active cysticercoids 24 days after the last immunization and were sacrificed 28 days after infection. Mice were challenged with *H. nana* by oral inoculation with 500 fresh eggs 24 days after the last immunization and were killed on day 22 after infection. Mice were challenged with *H. microstoma* by oral inoculation with three fresh active cysticercoids 24 or 34 days after the last immunization. These mice were sacrificed on day 26 or day 13, respectively, after infection. The methods used for experimental infection, examination for adult worms and determination of developmental stage have been described in our previous study [11].

Results

In the four control groups, four to 12 worms were found. No rats or mice immunized with non-viable *H. diminuta* or *H. nana* oncospheres were infected by the challenge organism (Tables 1 and 2). However, 30 of 40 mice immunized with non-viable *H. microstoma* oncospheres were infected on day 13 or day 26 after oral inoculation. From each experimental group, three to 12 worms were recovered. All six mice in the control groups were infected and 16 worms were recovered (Table 3).

Discussion

Subcutaneous injection of a crude homogenate of mature worms of *H. nana* with CFA or aluminum hydroxide evokes protection against egg challenge in immunized mice [13, 14]. Larsh reported a 75% reduction in cysticercoids recovered from immunized mice [13]. Coleman et al obtained more effective results, as indicated by almost complete

Table 1. Protection against *Hymenolepis diminuta* infection in rats immunized with non-viable homologous oncospheres

Rat group	*n*	Oncospheres injected/rat*	Rats infected	Total worms found
Test				
1	3	500 × 1	0	0
2	3	500 × 2	0	0
3	3	500 × 3	0	0
4	3	500 × 4	0	0
Control				
5	3	Vehicle	3	7

*Interval between successive immunizations was 1 week.

Table 2. Protection against *Hymenolepis nana* infection in mice immunized with non-viable homologous oncospheres

Mice (ICR) group	*n*	Oncospheres injected/mouse*	Mice infected	Total worms found
Test				
1	5	500 × 1	0	0
2	5	500 × 2	0	0
3	5	500 × 3	0	0
4	5	500 × 4	0	0
Control				
5	5	Vehicle	5	11

*Interval between successive immunizations was 1 week.

Table 3. Effect of immunization with non-viable *Hymenolepis microstoma* oncospheres in mice on worm recovery after challenge infection

Mice (ICR) group	*n*	Oncospheres injected/mouse*	Last immunization to challenge	Day infection assessed	Mice infected	Total worms found
Experiment 1			24 days	Day 26		
Test						
1	5	500 × 1			5	10
2	5	500 × 2			5	11
3	5	500 × 3			5	10
4	5	500 × 4			5	12
Control						
5	3	Vehicle			5	12
Experiment 2			34 days	Day 13		
Test						
1	5	500 × 1			5	6
2	5	500 × 2			5	11
3	5	500 × 3			3	3
4	5	500 × 4			5	6
Control						
5	3	Vehicle			3	4

*Interval between successive immunizations was 1 week.

failure of adult recovery [14]. In the present study, we demonstrated complete protection against infection by homologous parasites in rats or mice immunized with non-viable *H. diminuta* and *H. nana* oncospheres.

Mammalian intermediate hosts for larval cestodes have complete resistance to re-infection even with a single oncosphere [15]. In the present study, subcutaneous immunization with non-viable oncospheres also conferred protection on the definitive mammalian hosts of cestodes. Stimulation of complete resistance against *H. diminuta* and *H. nana* cysticercoids required one immunization with non-viable oncospheres. Repeated immunizations were not required.

We obtained very good results in rats or mice immunized with non-viable homologous *H. diminuta* or *H. nana* oncospheres. However, mice infected with *H. microstoma* were found in all four experimental groups after immunization with non-viable homologous oncospheres. Mice show a high level of resistance to challenge with *H. nana* cysticercoids only in the prepatent period, while mice that have been infected or in the patent period are highly resistant to both cysticercoids and oncospheres of *H. nana* [16]. Moreover, specific antibodies against oncosphere antigens have been demonstrated in mice with patent infection [17]. It is possible that inoculation with non-viable oncospheres may stimulate stage-specific immunity, which

may not protect mice from infection by homologous cysticercoids. However, further studies are required to clarify this suggestion.

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應用不具活性之同種六鉤幼蟲接種鼠類對抗包膜條蟲之感染

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接種同種或異種六鉤幼蟲(oncospheres)，可使豬體產生預防台灣條蟲之免疫反應。本研究之目的在確定接種不具活性之同種包膜條蟲(*Hymenolepis*)六鉤幼蟲可引起鼠類對縮小包膜條蟲(*H. diminuta*)、短小包膜條蟲(*H. nana*)及微小包膜條蟲(*H. microstoma*)之免疫力。六鉤幼蟲自蟲卵孵出，並儲存在 –70°C 超過一個月。實驗組包括四組 Sprague-Dawley 大白鼠或 ICR 小白鼠，每組各以皮下注射接種含有 500 個不具活性之六鉤幼蟲及 Freund 佐劑混合液一至四次(每次相隔一週)，控制組不接種六鉤幼蟲。接種後，每隻動物自口腔感染三隻新鮮具有活性之似囊尾幼蟲，並作解剖檢查。接種後之大白鼠或小白鼠不感染縮小包膜條蟲或短小包膜條蟲，惟 34 隻小白鼠有 28 隻感染微小包膜條蟲。結果顯示，鼠類接種縮小包膜條蟲或短小包膜條蟲可對同種包膜條蟲產生完全免疫力，對於已產生免疫力之宿主並不需要重覆接種。

關鍵詞：縮小包膜條蟲，短小包膜條蟲，微小包膜條蟲，接種，六鉤幼蟲

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