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Effective Protection Against Experimental *Taenia solium* Tapeworm Infection in Hamsters by Primo-Infection and by Vaccination with Recombinant or Synthetic Heterologous Antigens

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?1 ABSTRACT: The disease caused by *Taenia solium* is progressively being recognized as a growing global threat for public human health and pig husbandry that requires the development of effective control measures. A central participant in the taeniasis/cysticercosis transmission network is the human carrier of the adult tapeworm because of its great potential in spreading the infection. Herein, evidence is presented that a primary infection of golden hamsters with orally administered *T. solium* cysticerci improved the host's resistance against a secondary infection. Likewise, previous vaccination increased the hamster's resistance. Similar high levels of protection (>78%) were induced by systemic or oral vaccination with the S3Pvac anticysticercosis synthetic peptide vaccine or the highly immunogenic recombinant chimera based on the protective peptide KETc1 bound to *Brucella* spp. lumazine synthase (BLS-KETc1). Increased resistance after primo-infection and vaccination possibly results from changes in the immune conditions prevailing in the host's intestine. The contribution to protection from the KETc1 and BLS epitopes in a chimeric vaccine is under study. Preventive vaccination of definitive hosts of *T. solium* against the tapeworm, the most relevant step in the taeniasis/cysticercosis transmission, may greatly impact the dynamics of endemic disease and has not been studied or tried previously.

The metacestode stage of several cestodes, e.g., *Taenia solium*, *Taenia saginata*, *Taenia multiceps*, *Taenia crassiceps*, and *Echinococcus granulosus*, lodges within the tissues (e.g., brain, muscle, heart, liver, and lungs) of their intermediate hosts, e.g., pigs, cattle, sheep, rodents and goats, causing a condition called cysticercosis that produces great damage to animal husbandry throughout the world and threatens human health, most importantly in developing countries (Tanowitz et al., 2001). Controlling the transmission of *T. solium* is a subject of growing global concern and debate (Pawlowski et al., 2005) and has prompted the development of numerous technologies aimed at their diagnosis, treatment, and prevention (Sciutto et al., 2003). A few vaccines have been developed to protect the intermediate hosts with the aim of interrupting transmission (Huerta et al., 2001; Flisser et al., 2004; Manoutcharian et al., 2004). However, only in 1 vaccine (S3Pvac), has efficiency and suitability been assessed in the field against natural *T. solium* pig cysticercosis, and sustained and simultaneous additional control measures were found to be necessary to interrupt established transmission cycles. A key factor in the transmission of *T. solium* cysticercosis is the tapeworm carrier, a single worm of which may spread millions of the parasite eggs, each with the potential of developing into a cysticercus upon ingestion by suitable intermediate hosts (Gemmell and Johnstone, 1977).

In spite of the relevance of intestinal taeniasis in the dynamics of transmission, there are a few publications related to vaccination against the adult tapeworm stage of several cestodes (Andreassen, 1991), but not a single publication for *T. solium*. Although there is no factual evidence for effective immunity against adult tapeworms in humans, hints that it may occur derive from (1) the scarcity of tapeworm carriers amongst hundreds or thousands of intermediate hosts, in highly endemic conditions may in part result from differential effective immunity favoring tapeworm cysticerci (Martinez-Maya et al., 2003); (2) the extremely specific host–parasite relationships of *T. solium* and humans is suggestive of the stringency of immune recognition; (3) the breaching of host specificity in the golden hamster–*T. solium* model by pharmacological immunodepression is a more direct sign of immune involvement (Verster, 1971); and (4) the crippling in vitro effect of antibodies upon the ability of cysticerci to transform into tapeworms in immunodepressed hamsters (Garcia et al., 2001).

Niclosamide, praziquantel, and albendazol are variably effective against several helminths, including *T. solium*, and are the drugs most widely used in mass treatments (Tanowitz et al., 2001; Jeri et al., 2004). However, even if a highly effective treatment against taeniasis is developed, prevention of the establishment of adult tapeworms is the best approach, because it may greatly curtail the spread of millions of eggs, thereby preventing the development of cysticercosis in intermediate hosts.

Thus, we decided to explore the effect of vaccination against the adult tapeworm stage of *T. solium*. We used the golden hamster model developed by Gnezdilov (1957), which has proved to be useful in several laboratories to study the early stages of cysticercus development into adult parasites (Verster, 1971; Merchant et al., 1998). It is important to note that cysticerci collected from different pigs differ in their ability to develop into tapeworms (Garcia et al., 2001). This problem was minimized by selecting those experiments in which hamsters were infected with cysticerci, for which parallel cysticerci collected from the same pig showed more than 90% in vitro evagination after 24 hr of preincubation by using 10% pig bile in RPMI 1640 solution at 37 C. Also, a group of hamsters treated with saline (control group) was included in each infection experiment to verify the cysticercus transformation capacity.

To determine the most convenient time conditions to assess the vaccine's effects against taeniasis, 15 male and 15 female, 6- to 8-wk-old, not immunosuppressed hamsters were orally challenged with 6 cysticerci; all larvae used for the challenge were collected from a single naturally infected pig. Five of these males and 5 females were killed with CO_2 at 2, 3, and 4 wk postexposure (PE), and the number of tapeworms in each hamster's intestine was counted. The intestine was excised in its entirety and longitudinally opened with a pair of blunt-ended dissecting scissors. All recovered parasites were confirmed under a stereomicroscope. As shown in Figure 1, similar numbers of tapeworms were recovered from females and males at 14 days PE. Afterward, the number of tapeworms significantly declined in male, but not in female, hamsters, i.e., 7 of 10 males had no parasites, whereas all of the females were infected ($P = 0.03$). This is the first evidence of sexual dimorphism in the control of the intestinal *T. solium* tapeworms. Factors involved in this sexual dimorphism are presently under study. In all the following experiments the effect of the vaccine upon parasite load was assessed only in immunocompetent female hamsters.

We then attempted to determine whether the acquisition of immunity to experimental taeniasis by a first infection could prevent a second infection. Table I show results obtained after infection of 21 female ?2 hamsters. Seven hamsters (group 1) were challenged with cysticerci from a naturally infected pig, killed 16 days later, and the effectiveness

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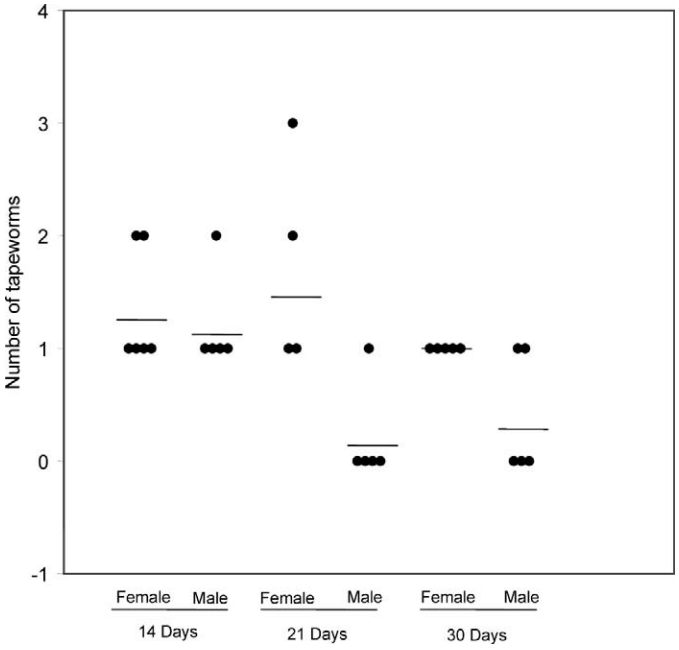

FIGURE 1. Individual number of tapeworms recovered from the intestine of golden hamsters at different times after infection with 6 *Taenia solium* cysticerci each. Bars represent the mean number of tapeworms per group.

of the first infection was confirmed. Thus, only 1 hamster had no tapeworms, whereas the rest carried 2–6 tapeworms each (3.1 ± 1.9). Of the other 14 hamsters, half of them (group 2) were treated with niclosamide and infected 10 days later with 6 cysticerci each. The remaining 7 hamsters (group 3) were first infected and then orally treated at day 10 PE and again exposed 10 days later with cysticerci from another naturally infected pig. A significantly lower number of tapeworms than that expected in the absence of primo-infection were recovered ($P = 0.05$).

The protective effect of primo-infection shown here in immunocompetent hamsters against subsequent experimental taeniasis points to the possibility of naturally acquired immunity against the intestinal tapeworm of *T. solium* and raises hopes of developing an effective vaccine.

Along with other factors, i.e., differences in pig and human susceptibility, naturally acquired immunity against taeniasis may contribute to the numeric disparity between the abundance of cysticercotic pigs and the scarcity of adult tapeworm carriers in highly endemic rural conditions (Fleury et al., 2003). This disparity would result if the transition of egg to cysticercus in the tissues is less vulnerable to the host's immune attacks than the cysticercus to tapeworm transition in intestinal lumen. The long-time residence of tissue cysticerci and their resistance to immune attack after their establishment are well recognized (Sciutto et al., 2003), but much less is known about intestinal immunity in taeniasis. The notion of acquired stage-differential natural immunity in *T. solium* disease is consistent with the 90% antigen-specific cell proliferation response coexisting with 10% of positive computed tomography scans and an extreme rarity of tapeworm carriers in the inhabitants of highly endemic rural areas of Mexico (Chavarria et al., 2003). We are unaware of any evidence in humans suggesting natural immunity to a secondary exposure to viable cysticerci, i.e., decreased likelihood of reinfection in treated and cured human cysticercosis than in the unexposed population, an important but difficult event to ascertain. Studies are under way to determine the role of systemic and local immunity in the protective effect of primo-infection.

Two different vaccines were tested in the taeniasis–hamster model. One vaccine was the field trial-tested S3Pvac anti-pig-cysticercosis synthetic vaccine (Huerta et al., 2001), which includes 3 peptides (GK-1, KETc1, and KETc12) originally derived from a *T. crassiceps* cysticerci cDNA library, which are present in the 3 different stages of the parasite, i.e., adult tapeworm, egg, and cysticercus, and are shared by *T. solium*.

TABLE I. Experimental primo-infection by *Taenia solium* tapeworm confers protection against a subsequent challenge

Group*	Treatment	No. of tapeworms† (mean ± SD)
1	Infection + ISS	3, 2, 3, 5, 0, 6, 3 3.1 ± 1.9
2	Niclosamide + infection	2, 0, 4, 6, 2, 1, 3 2.6 ± 1.9
3	First infection + niclosamide + second infection	0, 2, 0, 0, 1, 2, 1‡ 0.8 ± 0.8

* Hamsters in group 1 were orally infected and sacrificed 16 days afterward. Hamsters in group 2 were treated with nicosamide (chlorosalicylamide, Overoid, 250 mg/500 μl ISS/hamster) and infected 10 days later. Hamsters in group 3 were first infected and then treated with niclosamide (as described above). Ten days layer, they were reinfected and sacrificed 16 days afterward.
† Number of tapeworms recovered per hamster 16 days after challenged with 6 cysticerci each.
‡ Significantly different from group 2 ($P = 0.05$, 1-way ANOVA).

The other vaccine was the highly immunogenic recombinant BLS-KETc1 chimera, based on KETc1 expressed as a recombinant protein bound to the polymeric lumazine synthase from *Brucella* spp. (BLS) (Sciutto et al., 2005). These immunogens were evaluated considering that they are present in different structures of the *T. solium* tapeworm, including its tegument, thus making it possible to induce specific immunity against this parasite stage (Sciutto et al., 2003).

Table II (experiment 1) shows the protection induced by simultaneous immunization with S3Pvac (subcutaneously administered, s.c.) and/or BLS-KETc1 (orally applied, oral) against experimental tapeworm infection. As a control, a group of identically infected hamsters was treated

TABLE II. Vaccination effectively protect against *Taenia solium* tapeworm infection.

Experimental conditions*	No. recovered tapeworms/ hamster†	Mean ± SD
Experiment 1		
Saline	6, 6, 5, 1, 2, 2	3.7 ± 2.3a
Niclosamide	0, 0, 0, 0, 0, 0, 0	0b
BLS-KETc1/S3Pvac	0, 0, 0, 0, 0, 0, 0	0b
Experiment 2		
Saline	6, 6, 4, 1, 2, 0, 5	3.4 ± 2.4a
Saponine	5, 3, 4, 6, 3, 6	4.5 ± 1.4a
1. BLS-KETc1/S3Pvac	2, 0, 2, 0, 1, 0, 0	0.7 ± 0.9b
2. BLS-KETc1/S3Pvac	2, 2, 0, 2, 1, 1	1.3 ± 0.8b
3. S3Pvac/saline	1, 4, 0, 0, 0, 1	1.0 ± 1.5b
4. BLS-KETc1/saline	0, 0, 0, 2, 0, 1	0.5 ± 0.8b

* In experiment 1, hamsters were orally immunized with BLS-KETc1 (50 μg/200 μl/hamster) and 6 days later they received an s.c. booster of S3Pvac (15 μg/hamster of each peptide plus 50 μg saponine/100 μl ISS). All hamsters were orally challenged 5 days after the last immunization. A group of hamsters treated 10 days after challenge with niclosamide was included for comparison of a conventional effective treatment drug with the effects of the vaccines. In experiment 2, the relevance of oral and s.c. immunization was studied. Groups 1 and 2, immunized with BLS-KETc1 plus S3Pvac 6 days later and challenged 4 or 14 days after the last immunization, respectively. Groups 3 and 4, s.c. immunized with S3Pvac once or orally with BLS-KETc1 and challenged 10 days later. Controls were injected with saline or saponine alone at the respective times. All hamsters were challenged with cysticerci from the same naturally infected pig.
† Individual number of recovered tapeworms per hamster 2 wk after challenge with 6 cysticerci each.
‡ Mean ± SD followed by different letters indicate significant differences between groups ($P < 0.05$, Dunn's multiple comparison test).

10 days after challenge with niclosamide to compare the relative potency of the vaccines against conventionally effective antihelminthic drug. The data show that immunization with a combination of both vaccines, BLS-KETc1 oral, and followed by an s.c. booster of S3Pvac, induced total protection, just as did niclosamide.

To examine the effectiveness of the oral BLS-KETc1 versus the s.c. S3Pvac, an additional experiment was performed. Table II (experiment 2) shows a significant difference in the odds ratio (OR = 6.2, P = 0.015; OR, a statistic that compares differences in the risk of an event between 2 different groups by their quotient; Bland, 2000) of totally resistant hamsters between the control groups (1/13) and those vaccinated (12/25) in favor of hamsters that were vaccinated. Table II (experiment 2) also shows that a single dose (groups 3 and 4) of each vaccine, S3Pvac (s.c.) or BLS-KETc1 (oral), significantly reduced the parasite loads from the numbers observed in the respective controls as well as with the combined vaccines (groups 1 and 2). That there is no significant difference in parasite loads between groups 1 and 2, which differ in time interval between vaccine and challenge, shows that the protective effects of vaccination last for 14 days. Because both vaccines seem to be equally effective, the orally administered needle-free version (BLS-KETc1) would be preferable because it avoids costly logistic problems to make the vaccine suitable for mass immunization and it is also more acceptable because it is less invasive.

Our results show that the development of adult *T. solium* may be prevented by vaccination and may lead to the design of a potent tool to add in the control programs against taeniasis/cysticercosis transmission. In addition, successful vaccination against tapeworm infection may be an important contribution in the prevention of other cestodiasis. Indeed, the induction of effective immunity is well documented in rodent experimental hymenolepid infections, but the prospects-inducing effective immunity against *Echinococcus* spp. seems less promising (Andreassen, 1991). Perhaps the limited and varied success in doing so could be overcome by using more potent antigens, alternative immunization routes, or new antigen delivery systems.

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