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Genetic Vaccination against Murine Cysticercosis by Using a Plasmid Vector Carrying *Taenia solium* Paramyosin

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A plasmid vector carrying the immunoprotective amino-terminal fragment of *Taenia solium* paramyosin (VW2-1) was designed for genetic vaccination studies. Mice that were genetically immunized with VW2-1 and challenged by intraperitoneal inoculation of *Taenia crassiceps* cysticerci showed 43 to 48% reductions in the parasite burden, values which were similar to values obtained previously when the recombinant protein was used.

Human and porcine cysticercosis caused by *Taenia solium* is still prevalent in several countries of Latin America, Africa, and Asia (11). A strong effort is currently being directed toward the development of an effective vaccine against porcine cysticercosis. A number of strategies have been proposed, including the use of parasite crude extracts (9, 17), recombinant proteins (2, 3, 15), synthetic peptides (4), phage display (8), and genetic immunization (10, 18). Genetic vaccines are particularly appealing for applications in developing countries as they can be inexpensive to produce and store (5). In the present study, we evaluated the use of genetic immunization with the amino-terminal fragment of *T. solium* paramyosin (VW2-1), which was previously shown to be protective (15), as an alternative strategy for vaccination in the murine model of *Taenia crassiceps* cysticercosis.

A plasmid construct encoding a synthetic sequence for VW2-1 was designed for genetic immunization. The codon usage of the wild-type coding sequence for VW2-1 was adapted for mammalian cells (synVW2-1) by using procedures for gene synthesis (1). Briefly, a set of overlapping 60-mer oligonucleotides were assembled by PCR to construct synVW2-1, which was inserted into the pCMV plasmid vector (14). The construct was confirmed by DNA sequencing before large-scale purification with a QIAGEN Endofree Plasmid Giga kit (QIAGEN). A comparison of the wild-type and synthetic sequences of VW2-1 showed substantial changes in first (11.2%), second (4.85%), and third (52.6%) positions of codons. An extensive comparison of the outcomes of the immune responses elicited in mice genetically immunized with wild-type VW2-1 and with synVW2-1 consistently showed higher antibody and cellular immune responses with the synthetic gene (data not shown). The synthetic coding sequence is available from us upon request.

Vaccination assays were carried out by using 8- to 10-week-old female BALB/c mice that were bilaterally inoculated in the tibialis anterior muscle with pCMV-synVW2-1 or pCMV blank vector (100 μg of DNA per mouse in 100 μl of 0.9% saline). A third group contained naïve mice. After 2 and 4 weeks, mice were boosted with identical doses of plasmid DNA.

In order to evaluate if the antibodies raised by genetic immunization were cross-reactive with paramyosins of *T. solium* and *T. crassiceps*, Western blot assays were carried out as described previously (16). Briefly, nitrocellulose membranes were blotted with recombinant full-length TPmy (recTPmy) and recombinant VW2-1 (recVW2-1) or with crude protein extracts from *T. solium* and *T. crassiceps* cysts (6). Membranes were reacted with pooled mouse sera that were collected 45 days after genetic immunization. Blots were developed by using a peroxidase-conjugated rabbit anti-mouse immunoglobulin G(H+L) (Zymed) secondary antibody, followed by diaminobenzidine (Sigma). A single 98-kDa protein, a size consistent with the expected size of paramyosins, was recognized in both crude extracts (Fig. 1). Moreover, recTPmy and recVW2-1 were also recognized by the sera from genetically immunized mice. These findings are consistent with the antigenic similarity between the two species of taeniids (12), as well as with the cross-reactivity of paramyosins from distant invertebrate sources (7).

The cellular immune response elicited in genetically immunized mice was characterized by proliferation assays and cytokine production by using spleen cells cultured in vitro as described previously (15). Briefly, a pool of spleen cells from each group, obtained 45 days after immunization, was cultured in the presence of 1 to 5 μg of recVW2-1 per ml for 72 h to a density of 5×10^5 cells per well. The responsiveness of spleen cells to concanavalin A was also checked by stimulation with 1 μg of concanavalin A per ml. Eighteen hours before harvesting, cells were pulsed with [*methyl*-^{3}H]thymidine (1 μCi/well; NEN Life Science), and the amount of incorporated radioactive label was measured with a liquid scintillation counter (Betaplate, Turku, Finland). The results of proliferation studies showed that significant stimulation indices were obtained for

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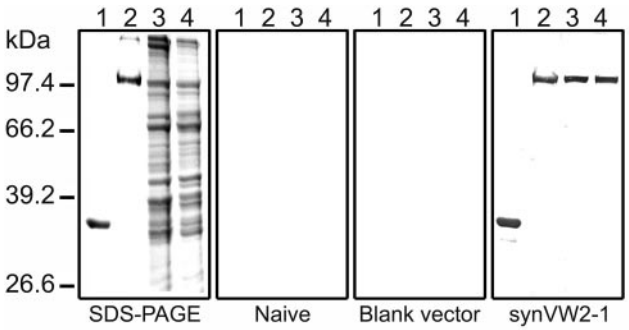

FIG. 1. Recognition of *T. solium* and *T. crassiceps* paramyosins by sera from mice that were genetically immunized with pCMV-synVW2-1. Samples of recVW2-1 (lane 1), recTPmy (lane 2), and crude extracts from *T. solium* (lane 3) and *T. crassiceps* (lane 4) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie blue (SDS-PAGE panel) or transferred to nitrocellulose membranes (Naive, Blank vector, and synVW2-1 panels). Blots were reacted with pooled sera from each group of mice and developed by using a peroxidase-conjugated rabbit anti-mouse immunoglobulin G(H+L) secondary antibody.

spleen cells from mice that were immunized with pCMV-synVW2-1 (Table 1). Spleen cells from naïve and immune animals showed similar responses to concanavalin A. The amounts of gamma interferon (IFN-γ) and interleukin-4 present in supernatants of spleen cells stimulated with recVW2-1 were determined by a sandwich enzyme-linked immunosorbent assay by using BD OptEIA cytokine kits (PharMingen). High levels of IFN-γ were detected in the supernatant of spleen cells from mice that were immunized with pCMV-synVW2-1 (Table 2). Assays with spleen cells from naïve and pCMV-inoculated mice showed that there was almost no IFN-γ and interleukin-4 production. The cellular immune response elicited in genetically immunized mice is consistent with a Th1 profile that has previously been related to protection in the murine model of cysticercosis (13).

The effect of genetic immunization with plasmid pCMV-synVW2-1 on a challenge with *T. crassiceps* cysts was evaluated in the murine model of cysticercosis as described previously (15). Two weeks after the last boost, mice were intraperitoneally challenged with 10 *T. crassiceps* cysts in saline, and they were sacrificed 25 days later. The cysts from the peritoneal cavities were collected and counted by individual, ignoring the experimental design. Genetic immunization with synVW2-1 resulted in 43 to 48% reductions in the parasite load (Table 3); these values were close to the 52% obtained with the recombinant product (15). No statistically significant differences were observed between the pCMV blank vector and naïve mouse groups.

Preliminary vaccination trials with pigs in which conventional or genetic immunization with recVW2-1 and synVW2-1 was used also produced about 50% reductions in the parasite load (data not shown). Recent vaccination trials with pigs in which the workers used a variety of strategies, including the use of oncosphere or metacestode crude antigens, synthetic peptides, recombinant proteins, phage display, and genetic immunization (2–4, 8–10, 17, 18), have resulted in claims of remarkably high levels of protection against *T. solium* cysticercosis. However, the intrinsic problems with carrying out experimental infection of pigs have meant that most groups have had to use small numbers of animals or low challenge doses in experiments. In the case of field trials under uncontrolled conditions, vaccines are required to show prophylactic as well as therapeutic properties. It appears that a multifunctional vaccine formulation that includes several protective antigens (on-

TABLE 1. Antigen-specific proliferation of spleen cells from genetically immunized mice

Stimulation[a]	Stimulation index[b]		
	Naïve	Blank vector	synVW2-1
RPMI[c]	1 ± 0.09	1 ± 0.37	1 ± 0.06
RPMI + recVW2-1	1.49 ± 0.15	0.99 ± 0.01	3.55 ± 0.61[d]
RPMI + ConA	22.89 ± 1.19	23.79 ± 0.18	21.62 ± 1.85

[a] Proliferative responses of spleen cells were determined by incorporation of [^{3}H]thymidine, after 72 h of culture in the presence of recVW2-1 (1 and 5 μg/ml) or concanavalin A (ConA) (1 μg/ml) as a control.
[b] Mean ± standard deviation, calculated from the means of triplicate samples for radioactivity incorporation after a 18-h pulse with [^{3}H]thymidine.
[c] The absolute values for RPMI-stimulated cells were 16.87 ± 1.43, 15.7 ± 5.76, and 16.93 ± 1.03 kcpm (means ± standard deviations) for the naïve, blank vector, and synVW2-1 groups, respectively.
[d] Absolute values for each experimental group were statistically different from the absolute values for RPMI-stimulated cells ($P < 0.05$).

TABLE 2. Cytokine profiles of spleen cells from genetically immunized mice

Spleen cells[a]	Production (pg/ml) of[b]:	
	Interleukin-4[c]	IFN-γ[c]
Naïve	≤7.8	≤31
Blank vector	≤7.8	≤31
syn VW2-1	≤7.8	705.66 ± 32.62[d]

[a] Spleen cells from different groups were cultured in vitro in the presence of recVW2-1 (5 μg/ml) to quantify interleukin-4 and IFN-γ in the culture supernatants.
[b] The values are means ± standard deviations for triplicate representatives of at least two experiments.
[c] The lower detection limits for interleukin-4 and IFN-γ were 7.8 and 31 pg/ml, respectively.
[d] Statistically significant difference between naïve cells and immune cells ($P < 0.01$).

TABLE 3. Effect of genetic immunization against murine cysticercosis caused by *T. crassiceps*

Expt	Immunization	No. of mice	Individual parasite loads (No. of cysts)	Avg load[a]	Load reduction (%)[b]
1	Naïve	9	52, 57, 75, 77, 80, 89, 98, 107, 109	83 ± 7	
	Blank vector	9	67, 75, 88, 89, 90, 95, 101, 101, 111	91 ± 5	
	synVW2-1	9	9, 13, 42, 44, 50, 58, 62, 65, 80	47 ± 8	43[c]
2	Naïve	8	65, 78, 90, 100, 102, 103, 105, 177	102 ± 12	
	Blank vector	9	45, 53, 60, 83, 89, 98, 107, 114, 116	85 ± 9	
	synVW2-1	9	35, 46, 47, 48, 49, 54, 59, 72, 72	54 ± 4	48[c]

[a] The values are means ± standard errors after 25 days of infection.
[b] The parasite load in naïve control mice was defined as 100% to calculate the effect of immunization on the parasite loads in other groups of mice.
[c] There was a statistically significant difference between naïve control mice and immunized mice ($P < 0.05$).

cosphere and metacestode) might be the way to produce a broad-spectrum vaccine to aid in the control of taeniosis/cysticercosis.

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