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Research note

Identification of CD4$^+$ T cell epitopes of *Taenia solium* paramyosin

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T cell mediated response is involved in a protective immune response against experimental cysticercosis conferred by immunization with Taenia solium *paramyosin (TPmy) to BALB/c mice. In this study, we analysed the TPmy amino acid sequence for predicted CD4$^+$ T cells epitopes. Five different regions of this protein showed that the residues anchor to bind the I-A^d molecule; synthetic peptides containing these epitopes were evaluated for their ability to induce lymphoproliferative responses of spleen cells from TPmy immunized mice. Among them, Tp176 (amino acids 176–192 sequence DDLQRQMADANSAKSRL) was the immunodominant T cell epitope of TPmy. Delineation of this epitope should facilitate analysis of the role of CD4$^+$ T cell response in experimental cysticercosis.*

Keywords Taenia solium *paramyosin, CD4$^+$ T cell epitope, cysticercosis, I-A^d*

Paramyosins are structural proteins of thick filaments in the muscle of several groups of invertebrates, including helminths, and in some cases they have been localized to tegumentry structures (1). Paramyosin is also an immunodominant antigen during infections caused by different flatworms such as *Schistosoma mansoni* (2), *Echinococcus granulosus* (3) and *Taenia solium* (4). Therefore paramyosin has also been used as a vaccine candidate against different parasitic diseases (5–7). Laclette and co-workers previously demonstrated that *T. solium* paramyosin (TPmy) provided a significant level of cross-protection in mice against a challenge infection with *T. crassiceps*. In addition, they showed that the NH_2-terminal region of TPmy induced a Th1-like immune response, suggesting the role of cellular immune response in the establishment of protective status against murine cysticercosis (8). In context it has been shown that administration of neutralizing antibodies against IFNγ to *T. crassiceps* infected mice during the early phase of infection rendered them more susceptible to cysticercosis (9). More recently using STAT6$^{-/-}$ mice it has been demonstrated that in the absence of the IL-4/IL-13 signalling pathway susceptible BALB/c mice developed a Th1 response that controlled parasite growth. In contrast, wild-type mice developed a Th2 immune response and remained susceptible to *T. crassiceps* (10). For these reasons identification of TPmy epitopes presented by MHC class II molecules and evaluation of their role in antigen presentation offers the possibility to understand and manipulate CD4$^+$ T lymphocyte response in murine cysticercosis. Here we have evaluated five potential I-A^d-restricted epitopes from TPmy.

BALB/c (H-2^d) female mice 4–6 weeks of age were used in all experiments. The animals were maintained in the animal care facility at CINVESTAV-IPN and treated according to international regulations for the care of laboratory animals. Recombinant TPmy was partially purified from transformed *Escherichia coli* BL21 inclusion bodies, solubilized in 5 M guanidine hydrochloride and then purified by affinity

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Table 1 Alignment of *Taenia solium* paramyosin 17-mers with binding motifs for I-A^d

	Sequence																
Position					**1**	2	3	**4**	5	**6** **A**	7	8	**9** **A/S**				
176–192	D	D	L	Q	**R**	Q	M	**A**	D	**A**	N	S	**A**	K	S	R	L
337–353	I	K	D	L	**Q**	A	E	**N**	E	**A**	L	A	**A**	E	N	G	E
376–392	T	V	E	I	**N**	T	L	**N**	S	**A**	N	S	**A**	L	E	A	D
379–395	I	N	T	L	**N**	S	A	**N**	S	**A**	L	E	**A**	D	N	M	R
431–447	A	N	R	R	**L**	T	D	**L**	E	**A**	L	R	**S**	Q	L	E	A

Peptides are aligned according to Ia anchors residues (bold).

chromatography through a HiTrap™ chelating column (Pharmacia Biotech). Purified protein was analysed by conventional SDS-PAGE and immunoidentified by Western blot using a specific polyclonal antiserum (8). TPmy peptides and 323–335-ovalbumin control peptide (11,12) were prepared using Fmoc (fluoroenyl-methoxicarbonyl) Synergy model 432 A synthesizer (Applied Biosystems, USA), according to standard procedures (13). The mice were immunized three times intraperitoneally (i.p.) at 10-day intervals, with 25 μg of TPmy in complete Freund's adjuvant (CFA, Sigma, St Louis, MO, USA) for the first immunization and in incomplete Freund's adjuvant (Sigma, St Louis, MO, USA) for the second and third immunizations. Another group of mice was injected once subcutaneously at the base of tail, with 100 μg of free peptide in CFA. Spleen cells or popliteal lymph node cells from immunized or control mice were obtained 10 days after the last inoculation and proliferation was assessed using a standard [^{3}H]-thymidine incorporation assay. Splenocytes were plated at 4×10^5 cells/well in 96-well flat bottom culture plates (Costar, NY, USA) and cultured in DMEM-10: DMEM (Gibco, NY, USA), 25 mM Hepes (Gibco, NY, USA), 50 mM Na_2HCO_3 (Sigma, St Louis, MO, USA), 50 μM 2-mercaptoethanol (Sigma, St Louis, MO, USA), 10% heat-inactivated foetal bovine serum (Gibco, NY, USA), 40 μg/mL of gentamicin (Sigma, St Louis, MO, USA) and 2 mM L-glutamine (Sigma, St Louis, MO, USA). Cells were stimulated with optimum concentration of TPmy peptide (titration was carried out previously) or with 2·5 μg/mL of Con-A (Sigma, St Louis, MO, USA). Plates were incubated at 37°C under an atmosphere of 95% air–5% CO_2 for 72 h. 1 μCi of tritiated thymidine (methyl-^{3}H TDR, Amersham, NJ, USA) was added to each well 18 h before harvesting onto glass filters. The samples were then counted on a β-scintillation counter (LKB-Wallac, Turky, Filand). Antigen-induced proliferation was expressed as counts per minute (cpm) and as means of triplicated wells. Alternatively, 5×10^7 splenic cells were labelled with 1 μM 5- (and 6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes, USA) according to Lyons and Parish (14) and cultured for 3 days with optimum concentration of TPmy or TPmy-peptides. Using standard protocols, stimulated cells were stained for FACS analysis in cold PBS containing 0·5% BSA and 0·05% sodium azide with allophycocyanin-conjugated anti-CD3, phycoerythrin-conjugated anti-CD4, CyChrome-conjugated anti-CD44 (all from Pharmigen, San Diego, CA, USA). The samples were acquired in a FACSCalibur (Becton Dickinson, San Diego, CA, USA).

Data were analysed with the WinMidi 2·8 software (//www.facs.scripp.edu/software by Joseph Trotter 2000).

Affinity chromatography TPmy was used for mice immunization during this study. The protein analysed by SDS-PAGE and Western blot revealed a characteristic 100 kDa band of paramyosin (data not shown). Peptides binding to I-A^d have a nonameric motif with four essential anchors, P1 (degenerate), P4 (aliphatic), P6 (alanine) and P9 (alanine) (11,15,16). Using this motif in the primary TPmy sequence analysis, we found that 17-mers Tp176, Tp337, Tp376, Tp379, and Tp431 (Table 1) possessed the binding anchors for I-A^d. It was decided to use the lymphoproliferative response to evaluate the antigenicity of those peptides. A strong proliferative response to Tp176, followed by a moderate response to Tp376 or Tp379 was observed. No response was obtained with the other peptides tested (Figure 1a). To measure proliferation of CD4$^+$ T lymphocytes in response to culture with those specific peptides, spleen cells from TPmy immunized mice were labelled with CFSE before culture with TPmy peptides or Ova control peptide. FACS analysis was carried out gating on CD3$^+$, CD4$^+$ and CD44^{hi} T cells allowing for analysis of antigen-reactive T cells. After 72 h of *in vitro* restimulation with TPmy two cell divisions were observed, whereas three cell divisions were induced in CD4$^+$ T lymphocytes stimulated with Tp176 and two cycles induced with Tp376 or Tp379. It is worthwhile to mention that the number of responding CD4$^+$ T cells to Tp176 were 2·3 fold more than those that

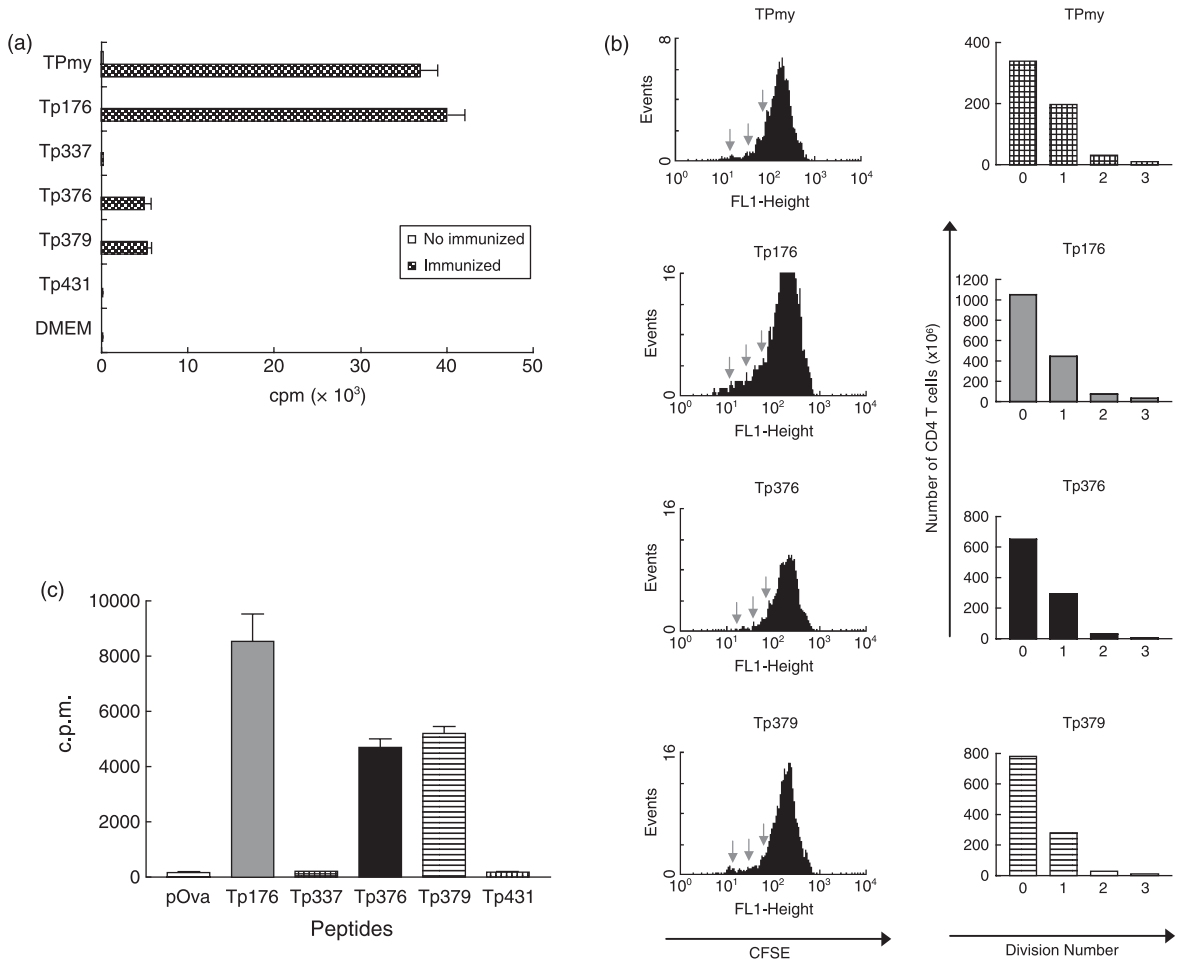

Figure 1 Tp176 is an immunodominant $CD4^+$ T cell epitope of TPmy. (a) Lymphoproliferative response of splenocytes from TPmy BALB/c immunized or non-immunized mice. 4×10^5 splenocytes were plated in triplicate in 96-well plates and stimulated *in vitro* with TPmy (10 μg/mL) or TPmy-peptides (1 μg/mL) for 72 h. Proliferation was measured by thymidine incorporation assay. These results are representative of three independent experiments and bars represent mean of triplicates. (b) Cell division of antigen-specific of $CD4^+$ T cells induced by stimulation with TPmy or TPmy-peptides. Splenocytes from TPmy BALB/c immunized and from non-immunized control mice were labelled with CFSE and stimulated for 72 h as described above. Then cells were stained with anti-CD3-APC, anti-CD4-PE, anti-CD44-Cy. 10^6 cells were acquired in FACSCalibur. $CD4^+$ T lymphocytes were gated and analysed on WinMidi software. Histograms show with arrows the divisions induced by TPmy or TPmy-peptides. Graphic bars show the $CD4^+$ T cells per million observed in each cell division. These results are representative of three independent experiments. (c) Comparison of lymphoproliferative response of popliteal lymph node cells from TPmy-peptides immunized mice. Responding lymph node cells were isolated from each group of TPmy-peptides immunized mice. 10^5 cells were plated in triplicate in 96-well plates and stimulated *in vitro* for 72 h with 1 μg/mL of the same TPmy-peptide used during immunization. For Ova-peptide stimulation cells isolated from Tp176 immunized mice were used. Proliferation was measured by thymidine incorporation assay. These results are representative of three independent experiments and bars represent means of triplicates.

reacted to TPmy, and 1·6 and 1·7 fold more than those that responded to Tp376 and Tp379, respectively (Figure 1b). To assess the immunogenicity of these peptides popliteal lymph node cells from TPmy-peptides immunized mice were restimulated *in vitro* with the same peptide used during immunization. A lymphoproliferative response to Tp176 as well to Tp376 and Tp379 was detected, but not to Tp337 and Tp431. The specificity of the immune response induced by TPmy-peptide immunization was tested using an $I\text{-}A^d$ restricted Ova peptide, which did not activate popliteal lymph node cells from Tp176 immunized mice (Figure 1c). Our results identified three different $I\text{-}A^d$ restricted epitopes of TPmy, and also showed a hierarchy between them; Tp176 was a clear immunodominant epitope whereas Tp376 and Tp379 behaved as secondary epitopes. These data are in agreement with Laclette and co-workers, who showed that T

cell responses in BALB/c mice were located near the amino terminus of TPmy (8). In addition they were similar to $CD4^+$ T cell epitopes described in other systems where an epitope hierarchy has also been observed (11,15).

Few reports about $CD4^+$ T cell epitopes derived from helminth antigens are available (17). Our knowledge about the cellular responses of parasitic diseases in human or experimental models is scarce. In cestodiasis, and particularly in cysticercosis, the information on $CD4^+$ T cell epitopes is null, in spite of it having been demonstrated that $CD4^+$ T cell response plays a critical role in experimental cysticercosis (9,10). In this context, the reactivity of Tp176 offers the possibility of manipulating the outcome of $CD4^+$ T cell responses in cysticercosis, for the development of a vaccine or to study the Tp176 specific $CD4^+$ T cell receptor repertoire analysis. Preliminary results suggest a possible Th1 response by IgG2a increase in serum from Tp176 immunized BALB/c mice. However, it is necessary to identify the cytokines profile produced by $CD4^+$ T cells specific to Tp176. Additionally, it would be interesting to define the residues critical for $CD4^+$ T cell activation and proliferation. In summary identification of prominent T cell epitopes from TPmy offers the possibility of understanding how the $CD4^+$ T cell response is involved in protection and how it is implicated in susceptibility to infection. In addition if we take into consideration that human and pig, the natural host of *Taenia solium*, mount a strong immune response against this antigen. The results observed using the mouse model might be also useful to understand the immune response against all type cysticercosis.

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