Evaluation of the protective potential of a *Taenia solium* cysticercus mimotope on murine cysticercosis

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**ABSTRACT**

An NC-1 mimotope from *Taenia solium* cysticerci can help identify patients with neurocysticercosis through immunoassay. After chemical synthesis, an NC-1 peptide was coupled to bovine serum albumin (NC-1/BSA) for use as an immunogen in murine *Taenia crassiceps* cysticercosis, which is an experimental model of cysticercosis caused by *T. solium*. NC-1/BSA immunisation decreased parasitaemia by inducing 74% protection compared to the 77% protection obtained with *T. crassiceps* crude antigen. The influence of immunisation was also observed on the size and stage of development of the parasite. Antibodies from NC-1/BSA-immunised mice recognised proteins from the tegument and from the buds, and intense immunostaining was observed in the final stage of the metacestode. The capacity of NC-1/BSA to induce protective antibodies which are reactive to proteins from the tegument of the metacestode suggests that this mimotope is a potential candidate for a vaccine against human and animal cysticercosis.

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1. Introduction

The metacestode stage (larvae) of *Taenia solium*, also known as *Cysticercus cellulosae*, is responsible for muscular and cerebral cysticercosis (neurocysticercosis [NCC]) in humans. The life cycle of *T. solium* includes pigs as intermediate hosts. Humans are the only known definitive host of the adult form, but they can act as accidental hosts through faecal-oral contamination with tapeworm eggs (hetero- or self-infection). Eggs hatch in the intestines, and the hexacanth embryos penetrate the intestinal mucosa, disseminate through the bloodstream, and lodge in muscle, soft tissue, and the central nervous system [1].

To develop new alternatives for serological NCC diagnosis, in 2009, our group used phage display biotechnology to find an amino acid sequence capable of identifying patients with NCC through indirect enzyme-linked immunosorbent assay (ELISA). We have demonstrated that, after chemical synthesis, the peptide NC-1 (SKSITITNKRKLTRK), a mimotope of *T. solium*, induced a humoral response in mice, in which antibodies recognised proteins from the scolex region during immunohistochemical study [2].

Considering previous studies about the capacity of phage-displayed peptides to induce protection against toxins [3], bacteria [4], viruses [5], fungi [6], and parasites [7,8], we sought to explore the potential of a synthetic peptide (NC-1), screened by antibodies against *T. solium* metacestode, to induce an immune response that could protect mice against murine cysticercosis. To provide more realistic tests for a vaccine candidate, a permissive host and a non-syngeneic (outbred) strain, as the genetically heterogeneous Swiss mice, was immunised with NC-1 coupled to BSA (NC-1/BSA) and challenged with cysticerci from *T. crassiceps*, and the capacity to induce protection was assessed as the reduction in worm burden [9].

Experiments with this Taeniidae are possible because its metacestode reproduces asexually by budding through intraperitoneal passage of mice [10], and it is usually used in immunological
and biochemical studies of cysticercosis [11–13] or as source of heterologous antigen in NCC immunoassays [14–16]. Therefore, murine cysticercosis was chosen as a model in our investigation because of the ease of maintaining T. crassiceps metacestode in the laboratory and measuring parasite loads without biohazard risks and because T. crassiceps and T. solium are phylogenetically related.

The results of our immunohistochemical studies revealed that the recognition profile of T. crassiceps cystercus by antibodies produced against NC-1/BSA corroborates the fact that T. crassiceps shares antigens with T. solium [13,16]. Furthermore, the protective potential of the NC-1 synthetic mimotope coupled to BSA indicated that synthetic mimotopes selected by phage display could be important vaccine candidates against parasites.

2. Material and methods

2.1. Animals

Seven to 8-week-old female Swiss mice were maintained at the Biotechnology and Production of Immunobiological Information Centre (CPPBi), Pirapora – PR, in accordance with guidelines of the local animal ethics committee. The animals were divided into 3 groups, each containing 8 or 9 mice. Both groups were given ad libitum access to food and water.

2.2. Antigen preparation

NC-1 was chemically synthesized including a terminal cysteine residue and coupled to bovine serum albumin (BSA) as described by Hell et al. [2]. BSA diluted in 20 mM sodium phosphate buffer (pH 7.4) containing 0.15 M sodium chloride was activated through reaction with sulfo-N-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Pierce Chemical Co., Rockford, IL), in concentration of 10 mg/mL. The solution was maintained at room temperature for 1 h under stirring. The excess reagent was removed with elution through a disposable PD-10 column. The activated BSA was reacted with the cysteine-containing peptide (5 mg/mL) at room temperature for 2 h under stirring and protected from light.

To stop the conjugation reaction, buffer containing 1 mM reduced cysteine was added. The peptide coupled to BSA was aliquotted and stored at −20 °C.

Crude antigen from an open reading frame (ORF) strain of T. crassiceps (TcCa) was maintained by consecutive passage of cysterci in the peritoneal cavities of mice and processed as described by Vaz et al. [14].

2.3. In silico analysis of the NC-1 sequence

The NC-1 amino acid sequence corresponding to SKSSITKRNLTRK [2] was analysed for sequence similarity to other sequences from Taeniidae species using the Basic Local Alignment Search Tool (BLAST) algorithm [17] on the National Center for Biotechnology Information public database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). In June 2011, each search was limited to just a single organism whose alignment had an E-value lower than 1.0. The following Taeniidae non-redundant (nr) sequence databases were accessed: T. crassiceps, T. solium, Taenia saginata, Taenia hydatigena, Taenia multiceps, Taenia pisiformis and Taenia taeniaeformis. The theoretical isoelectric point (pI) and molecular weight (Mw) of Taenia sp proteins were obtained from the Compute pI/Mw Program [18] at Expasy (http://expasy.org/tools/pi_tool.html).

2.4. Immunogen preparation and immunisation

In the first immunisation, mice were injected subcutaneously into the intra-scapular fold with one dose, i.e. 20 µg of NC-1 peptide coupled to BSA (NC-1/BSA), TcCa, or BSA dissolved in 50 mM phosphate buffered saline, pH 7.4 (PBS) and emulsified with complete Freund’s adjuvant (1:1, volume ratio) in a total volume of 100 µL. Following the guidelines of the animal ethics committee, the boost immunisation using the same route was avoided due to lesions caused by the complete Freund’s adjuvant, and at 2-week intervals, animals received new intra-peritoneal doses of immunogens emulsified with incomplete Freund’s adjuvant. One week after the fourth and eighth immunisation, approximately 50 µL of blood was collected from the mice by retro-orbital bleeding to measure antibody reactivity with ELISA.

2.5. ELISA evaluation of antibody reactivity

Plates with 96 wells (Falcon Labware, Oxnard, CA) were coated during 16 h at 4 °C with 10 µg/mL of the 3 antigens (non-coupled NC-1 peptide, TcCa, and BSA) dissolved in 50 mM carbonate buffer pH 9.6. After blocking with 2% (w/v) casein diluted in 50 mM phosphate buffered saline, pH 7.4 (PBS) and 0.05% (v/v) Tween 20, the mouse sera against each antigen diluted 1:100 in incubation buffer (Tween 20, 0.25% (w/v) casein) was added to each well and incubated at 37 °C for 1 h. The binding antibody was quantified using goat anti-mouse IgG (whole molecule)-horseradish peroxidase (Sigma # A4416) diluted 1:4000. The reaction was revealed using orthophenylendiamine and H2O2 and stopped by adding 20 µL of 2 N sulfuric acid. Absorbance readings (A492nm) were carried out in ELISA reader.

2.6. T. crassiceps metacestode infection

Following the protocol described above, mice were given a booster 1 week after the second blood sample was obtained. One week later, animals were infected with an intra-peritoneal injection of 5 cysticerci of T. crassiceps resuspended in 100 µL of PBS. Four weeks after this challenge, the animals were euthanised, and peritoneal washing in phosphate-buffered saline (150 mM NaCl, 10 mM sodium phosphate buffer and pH 7.2) was used to remove the cysticerci, which were counted and classified according to their stage of development (non-budding, larval, and final stage). Percent reduction of parasitaemia was calculated as follows: [(1−{mean worm burden of vaccinated group}/mean worm burden of BSA group)] × 100.

2.7. Immunohistochemistry

T. crassiceps metacestodes in the 2–3 mm larval stage (characterised by buddings) and in the final stage of development (a non-budding opaque vesicle) [11] were taken from an unrelated infected mouse and fixed in 4% (v/v) paraformaldehyde for 20 min. After washing in PBS (2.7 mM KCl, 1.8 mM KH2PO4, 137 mM NaCl, 10 mM Na2HPO4, pH 7.2, 304 mOs/m kg H2O), the samples were embedded in Tissue-Tek OCT (Sakura), frozen with liquid nitrogen, and stored at −80 °C. The tissues were sectioned 7 µm thick using a Leica CM1850 cryostat (Leica Microsystems, Germany) and placed on slides prepared with a 2% solution of BioBond (EMS) in acetone for 4 min. The slides were then rinsed for 5 min in distilled water and air dried. Additionally, aldehyde radicals were blocked with 100 mM glycine for 2 min and washed with PBS. Non-specific sites were blocked for 30 min with 2% casein diluted in PBS and 0.1% (v/v) Triton X-100, and sections were incubated for 2 h with pool of sera from immunised mice diluted 1:50 in PBS containing 2% (w/v) casein. Unbound antibodies were removed
Fig. 1. Pair wise alignment of the deduced amino acid sequence of NC-1 with *Taenia* spp proteins. Bold letters denote the consensus sequences between the NC-1 peptide and the *Taenia* spp protein sequence from GenBank. The numbering below the alignment refers to the amino acid position in the protein sequence. *The same alignment results were obtained with other *Taenia* spp.

with 3 washes in PBS. Finally, Alexa 488 conjugated anti-mouse secondary antibodies (Invitrogen) were diluted 1:250 in PBS containing 2% (w/v) casein and incubated for 1 h protected from light at room temperature. For nuclear staining, 10 mM 4',6-diamidino-2-phenylindole was applied for 5 min. Samples preparations were examined using a Zeiss Axio Observer Z1 inverted microscope (Carl Zeiss, Germany). The fluorescent probe was excited at 488 nm with emission using the LP 505 nm filter (green channel). Single images were obtained with a monochromatic camera (AxioCam HRm, Carl Zeiss, Germany) using a 40× lens for differential interference contrast and fluorescence intensity. Finally, AxioVision LE software was used for image processing and for morphometric measurements in the Zeiss image format.

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was used for statistical analysis of the results, and the Tukey test was used for pair wise comparison of samples. The significance of the difference in frequency of initial-, larval-, or final-stage cysticerci among groups was determined with the Chi-square test. Mean parasite length between NC-1/BSA and TcCa immunised groups was compared by using Student’s t test. A value of p < 0.05 was considered statistically significant.

3. Results

3.1. Similarities between NC-1 peptide sequence and *Taenia* spp proteins

Using bioinformatic analysis, we compared the NC-1 sequence to primary sequences of *Taenia* sp proteins deposited in the National Institutes of Health GenBank database. The alignments indicated identity of NC-1 peptide to cytochrome c oxidase and nicotinamide adenine dinucleotide dehydrogenase (NADH), two mitochondrial proteins of the respiratory chain. Some matches with paramosin, a component of invertebrate muscles, were also observed (Fig. 1).

Although searches using short peptide sequences can be virtually identical and have generally high E-value, our alignments presented good E-values for cytochrome c oxidase subunit III that ranged from 0.004 (*T. crassiceps*) to 0.14 (*T. solium*). The NADH subunit IV matches had E-value ranging from 0.25 (*Trichinella spiralis*) to 0.77

3.2. Antibody reactivity after fourth and eighth immunisations

Serum samples were obtained after the fourth (first bleeding) and eighth immunisations (second bleeding), and were assayed against the 3 antigens (BSA, TcCa, and non-coupled NC-1). ELISA results revealed the presence of antibodies in all groups of mice; however, the reactivity of serum from animals immunised with TcCa were inferior compared to those of the other groups. Furthermore, antibodies produced against NC-1/BSA were capable of discriminating among the NC-1 peptide sequence and BSA (Fig. 2A).

ANOVA indicated that the difference in reactivity among the 3 groups was significant (p < 0.05) with respect to the 3 immunogens (BSA, TcCa, and NC-1/BSA). This result was interpreted as if the dissimilarity among the immunogens was not the same after the fourth and eighth immunisations. Thus, we complemented our analysis with a comparison of the means using the post hoc Tukey test. The inequality among the groups changed after the booster. The Tukey test showed that after the eighth immunisation, the mean antibody reactivity of the 3 mice groups was equal (Fig. 2B). These results indicate that at the time of challenge, the mice from 3 groups had the same immunisation status.

3.3. Induction of protection by the NC-1 peptide

To analyse the protective potential of the NC-1 peptide, mice were immunised with NC-1/BSA, TcCa (positive control), and BSA (negative control). One week after the last booster, mice, including the control group, were challenged with 5 small *T. crassiceps* cysticerci. Thirty days later, the mice were euthanised, and the cysts were counted. NC-1/BSA immunisation reduced the worm burden by an average of 74.2% compared to the negative control (Table 2). Similarly, in the group immunised with TcCa, protection reached 77.7%.

For improving the normality of variables, data from recovered cysticerci was transformed by the equation \(\sqrt{x + 0.5}\). Considering the mean number of cysticerci from each group, it was possible to verify that animals immunised with the NC-1/BSA peptide or with

| NC-1 peptide | SKSSITITNKLRTK |
| Cytochrome c oxidase subunit III (*T. crassiceps*) | ILLGSSITITAFHILLGG |
| Cytochrome c oxidase subunit III (*T. solium*) | VLLGSSITITAFHILLGG |
| NADH dehydrogenase subunit 4 (*T. crassiceps*) | VLIKSSING1NLIMIVV |
| Paramosin (*T. solium*) | TKSAHURDAKRLTDL |
| | DMAPRTT1KRT1G |

*(T. crassiceps).* Table 1 lists the sequence similarities among NC-1 peptide and *Taenia* spp proteins.
Table 1
Alignment of NC-1 peptide with amino acid sequences from Taenia spp proteins.

<table>
<thead>
<tr>
<th>Putative protein</th>
<th>Theoretical pl/Mw (kDa)</th>
<th>Taenia species (accession)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome c oxidase subunit I</td>
<td>6.0/60.5</td>
<td>T. taeniaformis (YP_004062137)</td>
</tr>
<tr>
<td></td>
<td>4.8/24.9</td>
<td>T. crassiceps (NP_066229.2)</td>
</tr>
<tr>
<td></td>
<td>4.4/24.6</td>
<td>T. pisiformis (YP_003434404.1)</td>
</tr>
<tr>
<td>Cytochrome c oxidase subunit I</td>
<td>4.9/24.3</td>
<td>T. hydatigena (YP_003001995.1)</td>
</tr>
<tr>
<td></td>
<td>4.9/24.4</td>
<td>T. multiceps (YP_003001970.1)</td>
</tr>
<tr>
<td></td>
<td>5.0/24.3</td>
<td>T. solium (NP_659225.1)</td>
</tr>
<tr>
<td>NADH dehydrogenase subunit IV</td>
<td>5.9/48.7</td>
<td>T. pisiformis (YP_003434407)</td>
</tr>
<tr>
<td></td>
<td>5.4/46.0</td>
<td>T. hydatigena (YP_003001998.1)</td>
</tr>
<tr>
<td></td>
<td>5.7/48.7</td>
<td>T. crassiceps (NP_066232.2)</td>
</tr>
<tr>
<td>Paramyosin</td>
<td>5.3/98.8</td>
<td>T. saginata (Q8T305.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. solium (AAT34289.1)</td>
</tr>
</tbody>
</table>

* Theoretical isoelectric point (pl) and molecular weight (Mw) from proteins were calculated based on primary structure by using Expasy Compute pl/Mw tool (www.expasy.org/tools/pi_tool.html).

* Sequence similarity was searched on June, 2011 using the NC-1 sequence (SKSITITNKRRLTRK) as a starting query sequence.

Table 2
Induction of protection against Taenia crassiceps infection by NC-1/BSA immunisation.

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Individual number of cysts</th>
<th>Parasites recovered (mean ± SD)*</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC-1/BSA</td>
<td>1,243, 0, 91, 60, 14, 0</td>
<td>58.4 ± 88.7</td>
<td>74.2</td>
</tr>
<tr>
<td>TcCa</td>
<td>143, 38, 0, 38, 134, 0</td>
<td>50.4 ± 64.1</td>
<td>77.7</td>
</tr>
<tr>
<td>BSA</td>
<td>361, 327, 293, 34, 156, 341, 135, 164</td>
<td>226.4 ± 119.5</td>
<td></td>
</tr>
</tbody>
</table>

* Recovery of cysticerci was performed on day 30 after challenge. One-way analysis of variance revealed no significant difference between the NC-1 peptide coupled to bovine serum albumin (NC-1/BSA) and Taenia crassiceps crude antigen (TcCa) groups. However, both groups were statistically different (p < 0.05) from the group immunised with BSA (negative control group).

3.4. Influence of immunisation on cysticerci development

Cysticerci in the mouse peritoneum were counted and classified according to length or diameter and developmental stage—i.e. initial or larval stage (absence or presence of buds, respectively) or final stage. The Chi-square test allowed us to verify that the stage of development of cysticerci recovered from mice immunised with NC-1/BSA was significantly different (p < 0.0001, Chi-square = 58) from that of the cysticerci from the negative control group (Table 3). The absence of immune protection allowed a high number of cysticerci to develop and persist, especially at the larval stage, a phase considered to be reproductive. As expected, in relation to developmental stage, the level of protection in the TcCa group was different from that in the BSA group (p < 0.0001, Chi-square = 16). These results indicate a significant association between each immunogen and the stage of parasite development.

The influence of immunisation on the cysticerci development was verified when the length or diameter of cysts was measured after classification (Fig. 3). Because of the high variation between parasite dimensions, they were separated into 3 groups: ≤1 mm, 1 x 5 mm, and ≥5 mm. The coupled peptide and the crude antigen induced resistance in mice and similarly prevented an increase in the size of the parasites when compared with control group. On the other hand, although NC-1/BSA immunised mice had a smaller number of larval cysticerci, animals exhibited a more pronounced number of ≤1 mm cysticerci than TcCa group (p < 0.005, Student’s test) meaning active reproduction. These results indicate that NC-1/BSA was not as efficient as TcCa in inhibiting budding.

3.5. Localisation of NC-1 immunoreactive proteins on T. crassiceps metacastodes

Mice serum containing antibodies produced against the synthetic mimotope NC-1/BSA, TcCa, and BSA were used to immunolocalise native protein(s) in metacastodes of T. crassiceps. We performed an indirect immunofluorescence on the larval and final stages of the parasite. Immunofluorescence staining of mouse anti-NC-1/BSA antibodies on the T. crassiceps larval stage showed that the reactive protein(s) was present in the tegument of the cysticerci and, lightly, in the parenchyma. The immunoreaction

![Fig. 3. Effect of immunisation on the number and on the size of Taenia crassiceps metacastodes recovered from infected mice.](chart)
occurred mainly on the surface of the tegument (Fig. 4I). Different reactivity occurred in response to the internal tissues with TcCa antibodies; although the labelling was predominantly tegument staining, proteins from parenchyma cells were also significantly reactive (Fig. 4H).

The reactivity profile changed when sections of the final stage of the metacestode were used. The immunofluorescence displayed after using antibodies produced against TcCa was homogeneous on both parenchyma and tegument (Fig. 5H). This homogeneity was also verified when anti-NC-1/BSA antibodies were assayed, but curiously, an intense staining pattern of all tissue components of the section occurred as well (Fig. 5I). As expected, no reactivity was detected in sections incubated with mouse anti-BSA antibodies used as a negative control when tested on either the larval (see Fig. 3G) or the final stage of the developing parasite (see Fig. 4G).

### Table 3

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Mice examined (n)</th>
<th>Total length (mm) (mean ± SD)</th>
<th>Cysticeri stage (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC-1/BSA</td>
<td>7</td>
<td>1.83 ± 1.09</td>
<td>240</td>
</tr>
<tr>
<td>NC-1/BSA × BSA</td>
<td></td>
<td></td>
<td>169</td>
</tr>
<tr>
<td>TcCa</td>
<td>7</td>
<td>2.02 ± 1.15</td>
<td>0</td>
</tr>
<tr>
<td>TcCa × BSA</td>
<td></td>
<td>2.49 ± 1.34</td>
<td>0</td>
</tr>
<tr>
<td>BSA</td>
<td>8</td>
<td></td>
<td>690</td>
</tr>
</tbody>
</table>

*The influence of NC-1 peptide coupled to bovine serum albumin (NC-1/BSA) immunisation on cyst development compared to that of Taenia crassiceps crude antigen (TcCa) immunogen had a Chi-square value of 6 and p = 0.015.*

4. Discussion

We have shown that NC-1 (SKSITITINKRLTRK) can identify human neurocysticercosis on ELISA because it was selected using phage display by antibodies produced against T. solium antigens. NC-1 has induced antibodies that recognised four ∼45-kDa isoforms in a 2D SDS-PAGE assay and specific regions of suckers and the tissue surrounding the cysticercus scolex in an immunohistochemical assay [2].

The approach of using a peptide screened using phage display through specific antibodies is based on the fact that selected amino acid sequences can be identical [19,20] or present physicochemical characteristics or spatial organisation similar enough to the original epitope [21,22] to induce an immunoprotective response. In reference to NC-1 peptide properties [2] and to several previ-

![Fig. 4. Immunofluorescence staining of the larval stage of the Taenia crassiceps metacestode. Sections of 7-µm thickness were incubated with control serum antibodies against bovine serum albumin (BSA; G), Taenia crassiceps crude antigen (TcCa; H), or NC-1/BSA (I) and with Alexa 488-labelled anti-mouse antibody. In both images (H and I), an intense fluorescence can be seen on the tegument, but only TcCa antibodies stained the parenchyma uniformly (H). Each row of images shows the same section with a visible light image obtained using a differential interference contrast filter (A, B, C) and revealed with 4,6-diamidino-2-phenylindole-stained nuclei (D, E, F). Arrowheads: parenchyma (p) and tegument (t). Scale bar = 20 µm.](image-url)
ous studies that have investigated the capacity of phage-displayed peptides to induce immunoprotection against toxins [3,23], bacteria [4], viruses [5], fungi [6], endo-[7,8] and ectoparasites [24] the aim of this investigation was to evaluate whether a T. solium NC-1 peptide would induce an immune response able to cross-protect mice against murine cysticercosis.

Taking into consideration the recent discussions about the use of murine infections with T. crassiceps metacestodes in studies about human and porcine cysticercosis [25,26], mice were immunised with NC-1 coupled to BSA and challenged with T. crassiceps cysticerci after all animals, including the controls, presented the same serum reactivity owing to repetitive booster inoculations. Compared to animals that received exclusively BSA as an immunogen, NC-1/BSA impaired parasitaemia. Numerically, this protection was not significantly different from that induced in the group immunised with TcCa, and both immunogens also influenced the stage of development and size of cysticerci. The statistical data indicate that NC-1 was not as efficient as TcCa in inhibiting budding, as demonstrated by the higher number of cysticerci in the initial stage. This result was not completely unexpected because NC-1 represents only 1 epitope, whereas TcCa is a miscellany of immunogenic proteins.

Some phage-displayed peptides are called mimotopes because they are not homologue sequences to the antigen but can induce antibodies that recognise the mimotope and the original antigen owing to conformational similarities between them. In our experiments, this reactivity can be seen in immunostaining images of the larval stage in which an anti-NC-1 antibody reaction occurred mainly on the surface of the tegument.

The tegument of platyhelminthes, including Cestoda and Trematoda, consists of 2 layers: an outer anucleated syncytium and an inner nucleated region composed of a muscular layer. The surface syncytium of T. crassiceps is rich in large mitochondria [27] and enzymes for mitochondrial energy metabolism, including cytochrome c oxidase and NADH dehydrogenase [28,29]. Although some further analysis is required to identify the protein that can be effectively mimicked by the NC-1 peptide, the alignment with proteins from Taenia sp deposited in the GenBank database showed some identity between NC-1 and sequences of cytochrome c oxidase subunit III, and subunit IV of NADH dehydrogenase.

Mutations in complex I have made Caenorhabditis elegans susceptible to oxidative stress and seem to interfere with the functions of other mitochondrial respiratory chain complexes [30]. These data indicate these proteins may be relevant for the survival of tapeworms because they maintain the redox balance and control the production of oxygen free radicals in cells. Therefore, the strong immunoreactivity shown by anti-NC-1 antibodies on the final stage of T. crassiceps is indicative of a possible defence strategy. Further experiments may help us understand how complexes from the inner mitochondrial membrane that are involved in metabolic functions could induce immunoprotection. A hypothesis to be tested is whether T. crassiceps metacestode can secrete these proteins. Studies of the excretory/secretory proteomes of larval forms from 2 platyhelminthes, Schistosoma mansoni and Echinostoma caproni, have described several enzymes, including NADH dehydrogenase found in the extracellular environment [31].

NC-1 locating at the cysticercus tegument or in excretory/secretory products favours its recognition by patient serum.
References

[22] Förster-Waldl E, Riemer AB, Dehok AF, Neumann D, Brässwig KB, Balz
[30] Grad JJ, Lembire BD. Mitochondrial complex I mutations in Caenorhabditis elegans produce cytochrome c oxidase deficiency, oxidative stress and vitamin
[32] Hayman JJ, Moriarty KM, Charleston WA, Heath DD. Resistance against Tae
[34] Woolard DJ, Gauci CG, Heath DD, Lightowers MW. Protection against hydatid disease induced with the IEGS vaccine is associated with conformational epi