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IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - SHORT COMMUNICATION

Neurocysticercosis serodiagnosis: mimotope-based synthetic peptide as potential biomarker

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Abstract

Herein, we evaluate a mimotope-based synthetic peptidenamed $NC_4$1 to diagnose neurocysticercosis (NC), a neglected parasitic disease and a major cause of epilepsy worldwide. $NC_4$1 synthetic peptide was evaluated to diagnose NC, and total saline extract from *Taenia solium* metacestodes (SE) was used as control. Serum samples from patients with NC ($n = 40$), other parasitic diseases ($n = 43$), and healthy individuals ($n = 40$) were tested. Diagnostic parameters such as sensitivity (Se), specificity (Sp), likelihood ratio (LR), and area under curve (AUC) were calculated using receiver operating characteristic (ROC) curves. The sequence from *T. solium* phosphoenolpyruvate carboxykinase (PEPCK) was used for epitope prediction, resulting in one high-scoring patch centered at residue L247. $NC_4$1 synthetic peptide reached high diagnostic performance (Se 97.5% and Sp 97.5%, LR+ 39 and AUC 0.997). Data from diagnostic parameters and in silico analyses proved the usefulness of $NC_4$1 synthetic peptide as a diagnostic marker for human NC.

Keywords ELISA · Epitope · Prediction · $NC_4$1 · Serodiagnosis · Synthetic peptide

Introduction

Cysticercosis is a neglected tropical disease (FAO/WHO 2014) caused by *Taenia solium* metacestodes that can develop in several organs or tissues, leading to neurocysticercosis (NC), the most common parasitic disease affecting the human central nervous system (Garcia et al. 2014).

NC global burden is not precise due to the necessity of neuroimaging to confirm infection; in addition, there are problems with the sensitivity and specificity of diagnostic tests, since crude antigens cross-react with antigenic determinants shared with other helminths (Nash et al. 2013). Focusing on these problems, several advances were made leading to the achievement of antigenic fractions (Nunes et al. 2017), recombinant antigens (da Silva Ribeiro et al. 2010; Noh et al. 2014; Hernández-González et al. 2017), and synthetic peptides (Noh et al. 2014) applied to NC diagnosis.

Our previous results clearly demonstrated the diagnostic potential of a phage-displayed mimotope to diagnose NC (da Silva Ribeiro et al. 2010). The aim of this study was to investigate a synthetic peptide based on the mimotope sequence ($NC_4$1) and evaluate its diagnostic performance to detect NC in serum samples, as well as predicting B cell epitopes using the sequence from *T. solium* phosphoenolpyruvate carboxykinase (PEPCK), the native antigen of a single chain fragment variable (scFv) antibody previously selected against the phage-displayed peptide $NC_4$1 (Ribeiro et al. 2013).

A peptide was synthesized based on a mimotope sequence ($NC_4$1) that originated from phage-display selections, as previously described (da Silva Ribeiro et al. 2010; Feliciano et al. 2016). The peptide consisted of two repetitive epitope motifs spaced by GGGS, with an N-terminal BSA (bovine serum albumin) coupling and an amide in the carboxy-terminal end.

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Material and methods

Serum samples

Serum samples from 123 subjects from Uberlândia, Minas Gerais State, Brazil, were tested and divided into three groups: Group 1 consisted of 40 patients with NC definitive diagnosis according to Del Brutto (2012). Considering neuroimaging findings, metacestodes were classified according to Sotelo et al. (1985) as viable, in early degeneration-active NC (n = 17) or completely degenerated-inactive NC (n = 23).

Group 2 was constituted of 43 patients who had other parasitic diseases, including *Ascaris lumbricoides* (n = 7), *Echinococcus granulosus* (n = 4), *Enterobius vermicularis* (n = 5), hookworm (n = 6), *Hymenolepis nana* (n = 4), *Schistosoma mansoni* (n = 3), *Strongyloides stercoralis* (n = 4), *Taenia* sp. (n = 6), and *Trichuris trichiura* (n = 4).

Group 3 consisted of 40 apparently healthy volunteers from an endemic area for cysticercosis, with no household contact with *T. solium* infection. In this group, individuals provided three fecal samples that tested negative, as described previously (Ribeiro et al. 2013).

Antigens and immunoassay

Total saline extract from *T. solium* metacestodes (SE) was used as a protocol control and was prepared as previously described (Ribeiro et al. 2013). An ELISA was performed as previously reported (Ribeiro et al. 2013) with some modifications. Briefly, high-binding polystyrene microplates (Nunc MaxiSorp, Waltham, MA) were coated with $NC_4$1 synthetic peptide (1 μg/well) or SE (5 μg/ml). Serum samples and the enzyme conjugate (peroxidase-goat anti-human IgG, Fc specific; Sigma, St. Louis, MO) were diluted in phosphate-buffered saline (PBS) containing 0.05% tween-20 (PBS-T). When testing $NC_4$1 synthetic peptide, dilutions were made in PBS-T and skimmed milk 5% (PBST-M). Plates were washed six times using PBS-T. Samples were tested in duplicates.

Considering our previous results (Ribeiro et al. 2013) indicating *T. solium* PEPCK (accession number D2U5C3) as the native antigen of a scFv antibody selected against the phage-displayed peptide $NC_4$1, the sequence of this protein was evaluated using in silico tools (EpiSearch and Pepitope servers), and analyses were performed (Feliciano et al. 2016).

Statistical analyses

Statistical analyses were done by calculating the lower limits of positivity (cut-off value), established for optimal sensitivity (Se) and specificity (Sp) using the receiver operating characteristic (ROC).The diagnostic parameters calculated were area under curve (AUC), Se, Sp and positive likelihood ratio (LR+) according to Ribeiro et al. (2013). Reactivity indexes (RI) were tested for differences using the Wilcoxon (W) or Student's t test (t) between $NC_4$1 and SE.

Results and discussion

The recognition pattern of the $NC_4$1 phage-displayed peptide was initially evaluated by the determination of specific IgG responses using NC infected individual sera (da Silva Ribeiro et al. 2010), which provided promising results. The synthetic peptide based on this phage-displayed sequence reached higher specificity values (97.5% in this study and 92.5% in the previous one) (da Silva Ribeiro et al. 2010).

Synthetic peptides are advantageous for diagnostic applications, since they are well defined, can be produced in large amounts, and are highly pure (List et al. 2010). Besides these facts, $NC_4$1 peptide presented extremely low levels of cross-reactions.

Some diagnostic tests to detect NC use crude antigenic preparations, which can result in low specific diagnostic tests due to cross-reactions with antigens from other helminthic species. New alternatives to circumvent the cross-reactivity associated with crude antigens, such as partly purified antigens (Nunes et al. 2017) and recombinant (da Silva Ribeiro et al. 2010) or synthetic peptides, are being developed. Some strategies used to obtain these biomolecules have a subtractive or a purification step in which cross-reactive antigens can be removed, resulting in minimal or no cross-reactivity, and therefore, improving the specificity for the diagnosis.

$NC_4$1 was coupled to BSA in one attempt to increase binding to ELISA plates, according to Gómara and Haro (2007). In this study, using samples from patients with NC (Group 1), the synthetic peptide showed more positive results (RI > 1.0) than SE (Fig. 1a), considering the selected cut-off, although median values were greater for SE ($W = -436.00$, $p = 0.003$).

In Group 2, only one sample (2.5%) from a patient infected with *H. nana* (1/4) tested positive when using the synthetic peptide $NC_4$1. When testing SE, the cross-reactivity rate reached 15%, which occurred due to samples from patients infected with *A. lumbricoides* (3/7), *E. granulosus* (1/4), *H. nana* (1/4), and *S. stercoralis* (1/4). In Group 2, there was no statistical difference when comparing RI ($W = 224.00$; $p = 0.134$).

When using $NC_4$1 in Group 3, only 2.5% (1/40) of samples were positive, while for SE, the number of false positive cases reached 17.5% (7/40). No statistical difference was observed when comparing RI ($t = 0.453$, $p = 0.653$).

Diagnostic parameters are presented in Fig. 1b–c. The best indexes were achieved using the $NC_4$1 peptide (97.5% sensitive and specific) as confirmed by the high likelihood ratio (LR+ = 39) and AUC (0.997) values.

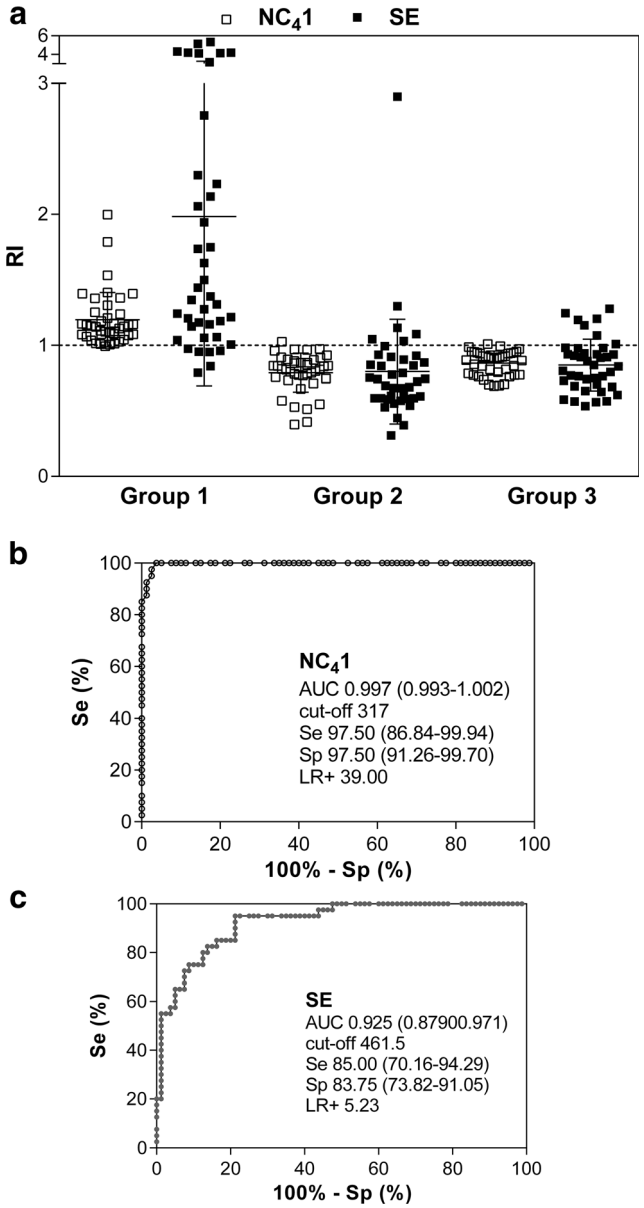

Fig. 1 Detection of IgG anti-*Taenia solium* metacestodes in serum samples from patients with neurocysticercosis (Group 1, $n = 40$), other parasitic infections (Group 2, $n = 43$), and apparently healthy individuals (Group 3, $n = 40$) was analyzed by enzyme-linked immunosorbent assay (ELISA) using the $NC_4$1 synthetic peptide or total saline extract (SE) from *T. solium* metacestodes. **a** Scatter dot plots. Dotted line indicates reactivity index (RI), positive samples: RI > 1; horizontal bars indicate median. B–C: ROC curve indicating area under curve (AUC), the optimum point of reaction (cut-off), sensitivity (Se), specificity (Sp), and positive likelihood ratio (LR+) (95% confidence intervals indicated)

The $NC_4$1 peptide sequence was mapped on the structure of *T. solium* PEPCK using EpiSearch, recognizing one high-scoring patch centered at residue L247, and the following match residues were found: M219, T222, T242, N243, M244, L247, T248, P249, L251, W254, M257, T294, P296, N297, W468, F482, and N484 (Electronic Supplementary Material 1). The EpiSearch program is a tool for predicting the possible location of epitopes on the surface of an antigen, which are ranked according to frequency of distribution of similar residues in the patch of mimotope input (Leung et al. 2017).

As PEPCK is a relatively conserved enzyme and there was no cross-reactivity between our peptide and serum from patients infected by *E. granulosus*, we also modeled (SWISS-MODEL) and tested in the Pepitope server (default mode; PepSurf algorithm) PEPCK from *Echinococcus vogeli* (accession number D2U5B4) and *E. granulosus* (accession number D2U5B7). Both *E. vogeli* and *E. granulosus* paths on a PEPCK 3D-structure with the highest $NC_4$1 peptide alignment score (T247, M249, N248, L252, T253, P254, L256, G258, W259, N228, and E232) differed from *T. solium* PEPCK path (N484, F482, N243, L247, T248, P249, L251, G253, W254, N223, E227A). In silico tools showed that our peptide can be recognized by specific circulating antibodies and has a different cluster from *Echinococcus* PEPCK, avoiding the most common cross-reactivity (with serum from patients infected with *Echinococcus*) in NC diagnosis.

Synthetic peptides mimicking relevant B and T cell epitopes are potentially ideal molecules for the development of reagents of diagnostic value (Noya et al. 2003), which also represent an interesting perspective to overcome problems observed in vaccine development, for example. Furthermore, studies have been performed using epitope prediction and synthetic peptides that mimic epitopes for diagnosing infectious and parasitic diseases such as strongyloidiasis (Feliciano et al. 2016), leishmaniasis (Lage et al. 2016), Chagas disease (Elisei et al. 2018), and fascioliasis (Meshgi et al. 2018).

In silico analyses and the diagnostic performance achieved in this study prove the usefulness of the $NC_4$1 synthetic peptide as diagnostic marker for human NC by using either ELISA assays or other diagnostic methods and systems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Research Ethics Committee of the Universidade Federal de Uberlândia (UFU),

Minas Gerais state, Brazil (approved under protocol number 041/09), and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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