Karolinska Institutet

Dept. Microbiology, Tumor and Cell Biology

Immunoreactive proteins in Taenia solium

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i MTC Lecture Hall, Theorells väg 1

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av

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ABSTRACT

Taenia solium cysticercosis is a neglected zoonotic disease that constitutes a serious public health problem in many low-income countries of Latin America, Africa, and Asia. Although diagnostic antigens are available, it is still necessary to identify new targets to improve the current diagnostic methods. Diagnostics tests are expensive, and most of them require the use of specialized equipment. Here, an expressed sequence tag (EST) library consisting of 5,760 sequences from T. solium cysticercus was constructed in which 1,650 unique sequences were identified, 845 of them previously unknown. Also, 2-DE was performed and succeeded in detecting several immunogenic proteins, among which TsolHSP36 and Tsol-p27 were identified by liquid chromatography-mass spectrometry (LC-MSMS), and Tsol-p27 was also found in our constructed EST library. A recombinant version of Tsol-p27 was produced and analysed by Western blotting, and it was recognized by sera from 13 NCC-positive humans but not by sera from control subjects. To develop a new and inexpensive diagnostic test that offers high sensitivity and specificity, we evaluated an immunodot blot assay using the previously analysed Tsol-p27 protein. The efficacy of the method was studied in comparison with the effectiveness of ELISA and of Western blot formats using the antigens TsolHSP36 and Tsol-p27. Compared to Western blot Tsol-p27, immunodot blot Tsol-p27 offered similar specificity (97.8% vs. 95.6%) but better sensitivity (86.7% vs. 76.4%). Also, sensitivity and specificity results were similar when comparing the ELISA and immunodot blot Tsol-p27 methods, and were lowest for Western blotting with TsolHSP36 (61.9% and 86.1%, respectively). Localization of Tsol-p27 was determined by immunohistochemistry using anti-rabbit Tsol-p27. Antibody response was observed in the parenchymal fold and tegument of the spiral canal. Sequencing analysis revealed that Tsol-p27 belongs to a group of proteins with the same bin/amphiphysin/rvs (BAR) domain as endophilin-B. This finding suggests that Tsol-p27 has functions related to membrane sorting, fusion, and transport, and that it may also aid growth of the parasite and support the adult tapeworm during colonization of the human intestine.

In conclusion, the present results show that the immunodot blot Tsol-p27 assay has good sensitivity and specificity, and it would be easy to implement this test in poorly equipped laboratories in endemic countries. Furthermore, this method is less expensive than ELISA and EITB analysis.

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