

Protein Expression Patterns In Adult *Angiostrongylus Costaricensis* Nematode

São Luiz, J.B.¹, Mota, E.M.¹, León, I.R.², Rebello, K.M.², Valente, R.H.², Perales, J.², Lenzi, H.L.¹ and Neves-Ferreira, A.G.C.²

¹Pathology and ²Toxinology Laboratories, Oswaldo Cruz Institute, FIOCRUZ, Av. Brasil 4365 CEP 21040-900, Rio de Janeiro, Brazil.

Email: jbsaoluiz@ioc.fiocruz.br

A.costaricensis is a Metastrongylidae worm widely distributed in Central and South Americas. In humans, it causes an intestinal acute inflammatory process named abdominal angiostrongyliasis, for which there is neither a diagnostic test nor a specific treatment. The interaction of parasites with the immune cells of the host occurs through surface or secreted proteins (1). This study aimed to determine the proteomic profiles of adult male and female helminths, including the identification of post-translational modifications and immunogenic proteins.

Adult worms were collected from *Sigmodon hispidus* rodents with 40 days of infection. Protein extraction was performed after maceration of the worms (20 mg) in 300 µL of 7 M thiourea, 2 M urea, 4% CHAPS, 40 mM tris base, 60 mM DTT and 1% v/v IPG buffer, followed by incubation at room temperature for 1 hour. After protein determination and TCA precipitation, samples were solubilized in the above described solution lacking tris base. Extracts of female or male adult worms were fractionated using 4-7 IPG strips followed by 15%T SDS-PAGE. Gels were developed, either with colloidal Coomassie or glycan-specific stain, or directly blotted onto a PVDF membrane probed with antiserum from mice experimentally infected with *A.costaricensis*. Protein identification of selected gel spots was done by mass spectrometry.

Male and female proteomic profiles were similar, with several spots recognized by mice hyperimmune serum preferentially located in the high molecular mass region of the gel. Protein identification was performed by MALDI-TOF/TOF MS after chemical derivatization with 4-sulphophenyl isothiocyanate (SPITC) (2), along with manual spectra interpretation and BLAST analysis. This strategy allowed confident identification of more than 70% of analysed proteins, which can be considered a very good yield for an organism with unknown genome. Most immunogenic spots were also glycosylated and included heat shock proteins, beta tubulin, protein disulfide isomerase, calreticulin, actin and galectin, among others. To date, very few biochemical analyses have focused on the nematode *Angiostrongylus costaricensis*, a medically relevant species in Latin America. Therefore, the proteomic study of this worm will certainly contribute to a better understanding of the parasite biology and pathogenesis, opening new possibilities for diagnostic and treatment.

Financial support: PDTIS -FIOCRUZ, CNPq and FAPERJ.