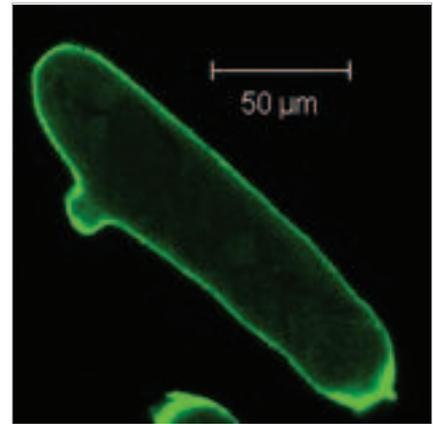


***Schistosoma mansoni* FUNCTIONAL GENOMICS APPLIED TO THE DEVELOPMENT OF VACCINES**

Luciana César de Cerqueira LEITE

Butantan Institute

The *Schistosoma mansoni* EST Genome Project, in São Paulo, Brazil, has recently generated 163,000 ORESTES (open reading frame-expressed sequence tags) from 6 stages of the parasite, resulting in 31,000 assembled gene fragments. It is estimated that this dataset represents 92% of a total 14,000 genes of the parasite, increasing 10-fold the number of ESTs available and increasing to 522 the number of complete genes of the parasite. Automatic annotation based on attributions within the Gene Ontology system, provided a vision of important biological functions of the parasite and permitted identification of a set of proteins with characteristics which could indicate some potential as vaccine candidates. The gene/ proteins were selected based on their attributed functions, which may indicate a potential role in the survival of the parasite in the host and possible exposure for interaction with the immune system. Of special interest were proteins with functions such as toxins, surface receptors for cellular adhesion, surface proteins and enzymes, and receptors for host factors. Expression of the genes in the stages interacting with the host was evaluated. Furthermore, the transcriptome of the parasite revealed that ~55% of the sequences coded for protein with no definable function. Part of these may be associated with adaptation to parasitism; within these, genes which have increased expression in transition between cercaria and schistosomula/ adult worms, could have interest as vaccine candidates. In parallel to the Transcriptome sequencing, we carried out a pilot project for characterization of the potential protective effect of some possible antigens identified in the beginning of the project, using DNA vaccines for presentation of the antigens to the immune system. This pilot project permitted establishing the basis of a Functional Genomics project for characterization of the protective potential of the identified genes. The present project proposes to investigate a selection of genes/proteins as potential vaccine candidates, presented as DNA vaccines. It will be organized in 3 subprojects with the following



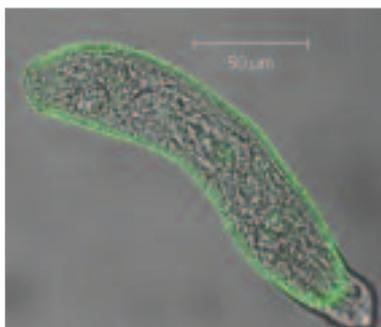
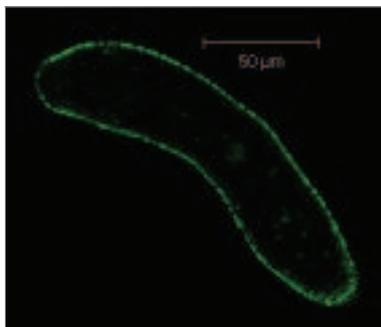
Immunolocalization of Dife5
Schisto 3h

objectives: 1) construction of DNA vaccines containing 25 candidate genes, characterization of the immune response induced against the respective antigens and evaluation of the protection induced against challenge with live cercaria; 2) characterization of the differential expression of the genes in the different life stages of the parasite by microarray using a panel of 4,000 selected genes; differential expression in parasites submitted to sera or cells from the immune system of immunized mice; 3) immunolocalization of the vaccine candidates in histological cuts of the parasite in the intra-host stages. This project should provide a first evaluation of the protective potential of a set of selected genes/proteins as vaccine candidates.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The São Paulo *Schistosoma* Genome Project generated an enormous amount of data from several stages of the parasite transcriptome. The data bank was used for an *in silico* identification of potential vaccine candidates, generating a list of genes/proteins with characteristics that could suggest their importance in the survival of the parasite, expression in intra-host stages and surface localization, which would allow interaction with the host immune system.

A preliminary screening evaluated the protection induced by DNA vaccines containing 30 of these genes, revealing a potential for 6 of these: a secreted exotoxin, Antigen 5, receptors for host factors, rVLDL and Stomatin, a surface enzyme, Apyrase, and 2 genes differentially expressed in the schistosomula stage. These antigens were submitted for patenting. Other antigens were selected from proteomic data.



Immunolocalization of Dife5
Schisto 7 day

Recombinant proteins were obtained for ~15 genes until now, allowing the investigation of different antigen presentations and the use of adjuvants, which may modulate the immune response induced towards conditions more favorable for the reduction of the parasitemia. The respective antibodies generated are being used to perform immunolocalization studies, confirming the surface localization of the antigens and their expression in intra-host stages. Furthermore, *microarrays* have been constructed: one with 4,000 gene fragments and the other with 44,000 oligonucleotides, representative of the parasite transcriptome. Parasites recovered from animals immunized with some of these antigens and protected against challenge have been analyzed as to the differential expression in relation to parasites recovered from control animals. On a whole these studies will allow a better evaluation of the potential of these antigens as vaccine candidates.

MAIN PUBLICATIONS

- Verjovski-Almeida S, DeMarco R, Martins EA, Guimaraes PE, Ojopi EP, et al. 2003. Transcriptome analysis of the acoelomate human parasite *Schistosoma mansoni*. *Nat. Genet.* **35(2)**:148-157.
- Verjovski-Almeida S, Leite LCC, Dias-Neto E, Menck CFC, Wilson RA. 2004. *Schistosoma* transcriptome: insights and perspectives for functional genomics, *Trends in Parasitol.* **20(7)**:304-308.
- Rodrigues V, Sá RG, Borges WC, Evangelista EA, Kettelhut IC. 2005. *Schistosoma mansoni*: Functional proteasomes are required for development in the vertebrate host. *Exp. Parasitol.*, **109**:228-236.
- Ribeiro-dos-Santos G, Verjovski-Almeida S, Leite LCC. 2006. Schistosomiasis - a century searching for chemotherapeutic drugs. *Parasitol Res.* **99(5)**:505-521.
- DeMarco R, Oliveira KC, Venancio TM, Verjovski-Almeida S. 2006. Gender biased differential alternative splicing patterns of the transcriptional cofactor CA150 gene in *Schistosoma mansoni*. *Mol. Biochem. Parasitol.* **150**:123-31.
- Pacifico, LG, Fonseca, CT, Chiari, L, Oliveira, SC. 2006. Immunization with *Schistosoma mansoni* 22.6 kDa antigen induces partial protection against experimental infection in a recombinant protein form but not as DNA vaccine. *Immunobiology.* **211(1-2)**, 97-104.
- Ojopi, EP, Oliveira, PS, Nunes, DN, Paquola, A, et al. 2007. A quantitative view of the transcriptome of *Schistosoma mansoni* adult-worms using SAGE. *BMC Genomics.* **8**:186-196.
- Levano-Garcia, J, Mortara, RA, Verjovski-Almeida, S, DeMarco, R. 2007. Characterization of *Schistosoma mansoni* ATPDase2 gene, a novel apyrase family member. *Biochem. Biophys. Res. Commun.* **352**:384-389.
- Verjovski-Almeida, S, Venancio, TM, Oliveira, KC, Almeida, GT, DeMarco, R. 2007. Use of a 44k oligoarray to explore the transcriptome of *Schistosoma mansoni* adult worms. *Exp. Parasitol.* **117**:236-245.
- Oliveira, SC, Fonseca, C, Cardoso, FC, Farias, LP, Leite, LCC. 2007. Recent advances in vaccine research against schistosomiasis in Brasil. Submitted to *Acta Tropica*.

Luciana César de Cerqueira LEITE

Instituto Butantan
Centro de Biotecnologia
Avenida Vital Brasil, 1500 - Butantã
05503-900 – São Paulo, SP – Brasil
+55-11-3726-9150
lcclite@butantan.gov.br