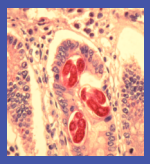
BERNHARD NOCHT INSTITUTE FOR TROPICAL MEDICINE
Tropical Medicine Section



Search for Secreted Immunomodulatory Proteins from *Strongyloides*

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The impact of the balance between host immune and parasite evasion mechanisms can be strikingly demonstrated in *Strongyloides stercoralis* infection: after decades of inapparent chronic infection maintained by low level autoinfection of immunocompetent individuals, treatment by immunosuppressive drugs or HTLV co-infection can disturb this balance and lead to disseminated strongyloidiasis with fatal outcome in most cases. Excretory/secretory (E/S) products allow the nematode parasite to skew the immune mechanisms and thereby allow its survival and propagation while multiple innate and adaptive immune responses control the parasite. Here we outline our search for immuno-modulatory secreted proteins of *Strongyloides ratti* closely related to the human parasite *S. stercoralis* using proteomic, molecular and immunological techniques.

1. Identification of secreted proteins by proteomics

The screening for E/S proteins in infective, parasitic and free-living *S. ratti* stages analysed by LC-ESI-MS/MS (collaboration: H. Steen, Harvard) provided 631 protein sequences (**Fig.1**).

Abundant major parasite-host-interacting secreted proteins are listed in **Table 1**.

Fig. 1. Distribution of secreted proteins from infective (iL3), parasitic and non-parasitic stages

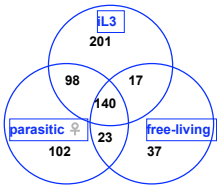


Table 1. *S. ratti* life cycle stage — **Major secreted Protein**

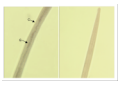
S. ratti life cycle stage	Major secreted Protein
Infective Larvae	• L3 Nie antigen (*S. stercoralis*) • Metalloproteases (AST) (O. volv.) • 24 kDa secreted proteins (*B.mal.)*
Parasitic females	• Prolyl endopeptidase (POP) (*T. d.*) • MIF (*A. duodenale*) • HSP 17 (*C. elegans*)
Free living stages	• Invapore (pore forming prot.) (*E.h.*) • Lysozyme fam. Prot. (*C. e.*) • E/S Mucin (*T. canis*)
All stages	• Galectins (*H. contortus*) • HSP10, HSP60 (*S. ratti)* • Thioredoxin peroxidase (*A.suum*)

2. Identification of gene sequences, gene structure and expression of selected proteins

(**Fig. 2., Table 2**)

- **Galectins**: *Sr*-Gal-1,2,3,5 (-11,21,22)
- **HSPs**: *Sr*-HSP 10, *Sr*-HSP 60, *Sr*-HSP 17
- **Proteases**: *Sr*-astacins AST, *Sr*-POP (Prolyl-endopept.)
- **Cytokine homologue**: *Sr*-MIF
- further (including novel) proteins will be characterized

Fig. 2.(A) Genome organization of *Sr*-HSP10 and -HSP60



Expression of *Sr*-HSP10 by *in situ* hybridization.

(B) Differential expression of candidate genes

RT-PCR with iL3, parasitic females and free-living stages

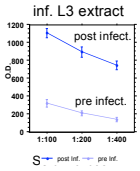
Stage	Mean Ct		
	Sr-HSP10	*Sr*-HSP60	*Sr*-GAPDH
iL3	27.34 ± 0.47	16.03 ± 0.04	13.62 ± 0.02
Parasitic female	21.62 ± 0.36	13.22 ± 0.09	13.47 ± 0.15
Free-living female	22.81 ± 0.8	14.36 ± 0.08	13.64 ± 0.04

3. Immune recognition of *S. ratti* secreted proteins by rat and human host IgG

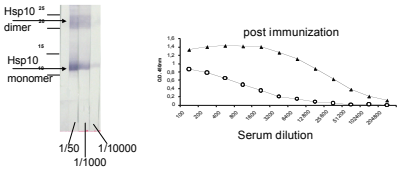
Recombinantly expressed proteins (*Sr*-Gal 3, *Sr*-HSP10, *Sr*-MIF) are recognized by IgG from rats infected with *S. ratti* or immunized with recombinant protein (*Sr*-HSP10) as well as by IgG from *S. stercoralis*-infected humans (ELISA,W-blots; **Fig. 3**).

Fig. 3. Host antibody recognition of *S. ratti* E/S proteins

(A) IgG response to iL3 extract after infection



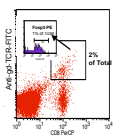
(B) High IgG levels after immunisation with *Sr*-HSP10

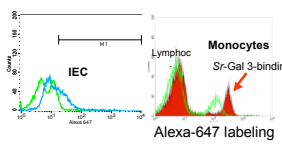


4. Interaction of *S. ratti* proteins with host immune cells (interactome)

First experiments indicate an interaction of *S. ratti* candidate proteins with rat and human host cells (**Fig. 4**). Ongoing and future studies with peripheral and mucosal innate immune cells (macrophages, neutrophils, eosinophils and epithelial cells) from rat and humans will elucidate whether selected E/S proteins exhibit immunomodulatory potency analysing receptor expression, protein release and apoptosis.

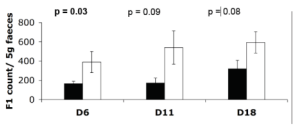
Fig. 4 (A) Detection of Foxp3+CD8+gd cells in the mucosa of rats immunized with *Sr*-HSP10





(B) Binding of *Sr*-HSP10/-Gal 3 (Alexa 647) **to intestinal epithelial cells (IEC) or monocytes**

(C) Effect of Sr-HSP10 immunization on the worm load after *S. ratti* infection



Conclusion: Multiple secreted proteins from *Strongyloides ratti* infective and parasitic stages are identified to be examined for immunomodulatory potency. Selected proteins including so far galectins, heat shock proteins, proteases and a cytokine homologue are currently characterised at genomic, proteomic and immunological level to finally evaluate the role of their homologues in the human pathogen *S. stercoralis*.