Immunolocalization of target schistosomal antigens using anti-cysteine proteases antibodies in murine schistosomiasis

Presented by

Alyaa Ahmed Farid Ahmed El-Said

M.SC (Immunology),
Zoology Department

A Thesis Submitted to Faculty of Science
For the Degree of
PH.D of Science
(Immunology)

Zoology Department
Faculty of Science
Cairo University
(2010)

بسم الله الرحمن الرحيم

إِنَّمَا يَخْشَى ٱللَّهَ مِنْ عِبَادِهِ ٱلْعُلَمَٰٓؤُا

إِنَّ اللَّهَ عَزِيزٌ غَفُور

صدق الله العظيم

Approval Sheet

Title of the PH.D Thesis

Immunolocalization of target schistosomal antigens using anti-cysteine proteases antibodies in murine schistosomiasis

Name of the Candidate

Alyaa Ahmed Farid Ahmed El-Said

This thesis has been approved for submission by the Supervisors: **Signature**

1- Pro. Dr. Abdel Hakim Saad

2- Pro. Dr. Ahmed Mohamed Osman

3- Dr. Ibrahim Rabee Shalash

1&2: Professors of Immunology
Zoology Department
Faculty of Science
Cairo University

3: Associate Professor of Immunoparasitology
Theodor Bilharz Research institute
Giza, Egypt.

Head of Zoology Department
Prof. Dr. Kouthar Abo El-Ela

ACKNOWLEDGMENT

First of all I express my deepest gratitude to God to whom I owed every thing I learned and every success in my life.

I would like to express my deepest gratitude to **Prof. Dr. Abdel Hakim Saad**, Professor of Immunology, Department of Zoology, Faculty of Science, Cairo University, for his generous help, supervision, his guidance and his kind help. Also, I would greatly appreciate the unfailing enthusiasm of the supervision during the writing and checking the final manuscript.

I wish to express my sincere thanks to **Prof. Dr. Ahmed Osman**, Associate Professor of Immunoparasitology, Department of Zoology, Faculty of Science, Cairo University, for his generous help, sincere effort, and continuous encouragement in supervising time research.

My special deep thanks and appreciation are also due to **Prof. Dr. Ibrahim Rabee**, Associate Professor of parasitology, Theodor Bilharz Research institute, to whom I am deeply indebted for having given me the chance to undertake the subject of this thesis, and whose guidance, supervision, stimulating suggestion, and endless support.

I am also more grateful to my family especially to my wonderful mother and my father for their support and encouragement through this time.

To my wonderful MOTHER who wanted to see me a doctor and helped me to reach to what I became now.

To my kind FATHER who was the first teacher in my life.

To a very special person in my life; my beautiful son SAIF.

ABSTRACT

Schistosomiasis is an important parasitic disease in many parts of the tropics, second only to malaria as a source of morbidity and mortality. Diagnostic methods include microscopic examination of excreta such as the Kato-Katz method, radiography, and serological techniques.

Proteases have been under scrutiny as targets of immunological or chemotherapeutic anti-*Schistosoma* agents because of their vital role in many stages of the parasitic life cycle. Upon the basis of the mechanism of peptide hydrolysis, five major protease classes are known: serine, cysteine, aspartic, threonine, and metalloproteases. Proteases of all five catalytic classes have been identified from *S. mansoni* through proteomic or genetic analysis.

In this study, the produced polyclonal antibodies (pAb) against *S. mansoni* cysteine protease (CP) antigens were labelled with horseradish peroxidase (HRP) conjugate and used to demonstrate the presence of a highly reactive CP epitopes in the different organs of *S. mansoni* naturally infected mice and its different life cycle stages. Also, pAb successfully detects CP antigens in stool and serum samples of *S. mansoni* infected patients by sandwich ELISA with a sensitivity of 92.8% and 90.47% respectively. Moreover, a positive correlation was observed between *S. mansoni* egg counts and both stool and serum antigen concentrations. Thus, it can be concluded that purified 27.5 kDa CP obtained from E/S products of *S. mansoni* worms could be introduced as a suitable candidate antigen for early immunodiagnosis of *S. mansoni* infected human using sandwich ELISA for antigen detection and serodiagnosis.

Key words:

S. mansoni
Cysteine proteases
Gel filtration chromatography
SDS-PAGE
Immunolocaliztion
ELISA
Immunoflurosence
Polyclonal IgG
Immunostaining

CONTENTS

LIST OF ABBREVIATIONS

A	**Central arteriole**
Ab	**Antibody**
Ag	**Antigen**
AMC	**7-amino-4-methylcomarin**
AST	**Antigenic sequence tags**
AWA	**Adult worm antigens**
BC	**Bowmen's capsule**
BD	**Bile duct**
BS	**Blood sinusoids**
BS	**Bowmen's space**
BSA	**Bovine serum albumin**
bSmCL1	**Bacterial SmCL1**
CAA	**Circulating anodic antigen**
CB	**Cathepsin B-like**
CCA	**Circulating cathodic antigen**
CCL	**Chemokine ligand**
CL	**Cathepsin L-like**
Con A	**Concanavalin A**
COPT	**Circumoval precipitin test**
CP	**Cysteine protease**
CT	**Computed tomography**
CV	**Central vein**
DAB	**Diaminobenzidine**
DC	**Dendritic cells**
DDIA	**Dipstick dye immunoassay**
DEAE	**Diethylaminoethyl**
DNA	**Deoxyribonucleic acid**
DT	**Distal convoluted tubule**
DTH	**Delayed-type hypersensitivity**

E/S	**Excretory/secretory**
EF-hand motifs	**Calcium-binding motifs composed of two helixes, E and F, joined by a loop**
EITB	**Electroimmunotransfer blotting**
ELISA	**Enzyme- linked immunosorbent assay**
epg	**Eggs per gram stool**
EST	**Expressed sequence tag**
FABP	**Fatty acid-binding protein**
FCS	**Fetal calf serum**
FDA	**Fast Dot ELISA**
FITC	**Fluorescein isothiocyanate conjugate**
G	**Glomerulus**
GAPDH	**Glyceraldehyde-3-phosphate dehydrogenase**
GC	**Gynaecophoric canal**
GL	**Gut lining**
Glc	**Glucose**
GST	**Glutathione S-transferase**
H_2O_2	**Hydrogen peroxide**
HA	**Hepatic artery**
HBV	**Hepatitis B virus**
HCV	**Hepatitis C virus**
HE	**Hematoxylin and eosin**
HEPES	**N-2-hydroxyethyl piperazine-N-2-ethanesulfonic acid**
HL	**Hepatic lobule**
hr	**Hour**
HRP	**Horseradish peroxidase**
HS	**Hepatic strand**
HSC	**Hepatic stellate cell**
HSP	**Heat shock protein**
HSS	**Hepatosplenic schistosomiasis**
i.m	**Intramuscular injection**
ID	**Intradermal test**

IFN-γ	**Interferon gamma**
IgG	**Immunoglobulin G**
IgG pAb	**IgG polyclonal antibody**
IHA	**Indirect hemagglutination assay**
IIF	**Indirect immunofluorescence**
IL	**Interleukin**
kDa	**Kilo Dalton**
LC	**Langerhans cell**
LDNF	**Fucosylated LacdiNAc**
LeX	**Lewis X**
LP	**Lamina propria**
LPS	**Lipopolysaccharide**
LR	**London resin**
Lutz/HPJ	**Lutz/Hoffman, Pons & Janer**
M	**Mucosa**
m	**Miracidium**
mAb	**Monoclonal antibody**
MEG	**Major egg glycoprotein**
MIFC method	**Merthiolate iodine formaldehyde concentration method**
MIP	**Mitochondrial intermediate protease**
ML	**Muscle layer**
mol. wt.	**Molecular weight**
MPP	**Mitochondrial processing protease**
MSA	**Major serological antigens**
MZ	**Marginal zone**
NO	**Nitric oxide**
NPV	**Negative predictive value**
OD	**Optical density**
OPD	**O-phenylenediamine**
Os	**Oral sucker**
pAb	**Polyclonal antibody**
PALS	**Periarterial lymphatic sheath**

PAS	**Periodic Acid Schiff reagent**
PBMCs	**peripheral-blood mononuclear cells**
PBS	**Phosphate-buffered saline**
PBS/T	**PBS/tween**
PCR	**Polymerase chain reaction**
PEPCK	**Phosphoenolpyruvate carboxykinase**
PI	**Isoelectric point**
PNA	**Peanut agglutinin**
PPV	**Positive predictive value**
PT	**Proximal convoluted tubule**
PTGS	**Posttranscriptional gene silencing**
PTK	**Protein tyrosine kinase**
PV	**Portal vein**
PZQ	**Praziquantel**
R	**Correlation coefficient**
RC	**Renal corpuscles**
RP	**Red pulp**
RPMI	**Roswell Park Memorial Institute**
RT-PCR	**Reverse transcriptase-PCR**
S. bovis	***Schistosoma bovis***
S. haematobium	***Schistosoma haematobium***
S. intercalatum	***Schistosoma intercalatum***
S. mansoni	***Schistosoma mansoni***
S. mekongi	***Schistosoma mekongi***
SBSP	**Schistosome Biological Supply Program**
SD	**Standard deviation**
SDS-PAGE	**Sodium dodecyl-sulfate polyacrylamide gel electrophoresis**
SE	**Saline extract**
SE2	**Reextraction of adult worms**
SEA	**Soluble egg antigen**
SEi	**Initial fraction of SE**
Ser	**Serine**

Sj22.6	***S. japonicum* 22.6**
SjCL1	***S. japonicum* CL1**
Sm	***S. mansoni***
Sm20.8	***S. mansoni* 20.8**
Sm21.7	***S. mansoni* 21.7**
Sm22.6	***S. mansoni* 22.6**
SmCB	***S. mansoni* Cathepsin B-like endopeptidase 1**
SmCE	***S. mansoni* cercarial elastase**
SmCL1	***S. mansoni* Cathepsin L-like**
SmDPP IV	***S. mansoni* dipeptidyl peptidase IV**
SmFes	**PTK in *S. mansoni***
SmPepM8	***S. mansoni* polypeptide M8**
TBRI	**Theodor Bilharz Research Institute**
TCA	**Trichloroanisole**
Teg	**Tegument**
TEM	**Transmission electron microscope**
Th1	**T helper cell 1**
Th2	**T helper cell 2**
Thr	**Threonine**
TLRs	**Toll like receptors**
TNF-α	**Tumor necrosis factor-α**
USG	**Ultrosonography**
Vs	**Ventral sucker**
WB	**Western blot**
wk	**week**
WHO	**World health organization**
WP	**White pulp**
0–3-h RP	**Released proteins by larvae 0-3 hr after transformation**

LIST OF FIGURES