

Schistosoma mansoni :
Purification and Characterization of
Schistosome-specific IgG Subclasses

Thesis

Submitted to the Faculty of Science

Ain Shams University

In partial Fulfilment of the Requirements
for the Degree of Master of Science

By

Mahmoud Yakout El-Shemerly

Demonstrator in the Department of Biochemistry

574,293
M Y

Supervisors

Prof. Dr. Ibrahim Hassan Borai

Professor in Biochemistry Dept.

Faculty of Science, Ain Shams University



Dr. Kamal Ali Fathy Shalaby

Lecturer in Biochemistry Dept.

Faculty of Science, Ain Shams University

Dr. Mohamed Karim Kamal

Medical Research Specialist

Head of Immunopathology Division

NAMRU-3

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا
إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

مَهْدَقَ اللَّهِ الْعَظِيمِ



BIOGRAPHY

Date and place of birth: 25.1.1968 Cairo
Date of graduation 1989
Degree awarded B.Sc. Biochemistry
Grade M.Sc. in Biochemistry
Occupation 1989
Date of Appointment 1991
Date of registration 1994

Supervisors

Prof. Dr. Ibrahim Hassan Borai
Dr. Kamal Ali Fatthy Shalaby
Dr. Mohamed Karim Ahmed Kamal

Head of Department

Prof. Dr. Nadia Mohamed Abdallah

Courses: Studied by candidate in partial fulfillment for
Master Degree of Science.

Language: English, M.Sc. course.

Examination passed on September 1990.

Biochemistry Courses:

1. Molecular biology and immunology.
2. Energetics.
3. Methodology.
4. Hormones.

Examination passed on September 1991.

ACKNOWLEDGEMENT

I would like to express my deep thanks and grateful to **Professor Dr. Ibrahim Hassan Borai**, Professor of biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University, for his tutorial guidance and active supervision.

Sincere appreciation and gratitude to **Dr. Mohamed Karim Kamal**, Medical Research specialists Immunopathology Division, NAMRU-3, for his continuous encouragement and great effort in planning supervision and support throughout the course of the thesis.

I would also like to express my gratitude to **Dr. Kamal Fath Shalaby**, Lecturer of Biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University for his help, supervision and efforts throughout the whole work.

My special thanks and regards to **Miss Hind Shaheen and Dr. Salwa Fouad** for their valuable cooperation and unforgettable help. I would like to express my deep thanks to all members of immunopathology division, NAMRU-3 specially **Sherif Helmy, Moustafa Abd El Faddeal and Mohamed Housny** for their kind help and sweet dealing and to all of my colleagues at the Biochemistry Department, Ain Shams University, for their support.

It is worth mentioning that this work was carried out at the Immunopathology Department NAMRU-3 and supported by the Naval Medical Research and Development command, Bethesda, M.d., work unit NO. 3M 162 770 A 870. AQ 126.

T O M Y

CONTENTS

	<i>Page</i>
- ACKNOWLEDGEMENT	
- ABBREVIATIONS	
- AIM OF THE WORK	1
- INTRODUCTION	
I. Schistosomiasis	1
II. Schistosome surface antigens	9
III. Immunoglobulin isotypes and protective immunity	18
a Immunoglobulin isotypes	18
IgA	19
IgE	21
IgM & IgG	24
IgG subclasses	25
b. Blocking and protecting antibodies	31
c. Passive immunization	35
 - MATERIALS AND METHODS	 41
Preparation of antigens	
1. Preparation of crude soluble egg antigen	41
2. Preparation of soluble schistosomal adult worm antigen	41
3. Preparation of Np-40 adult worm extract antigen	42
4. Preparation of Np-40 3 hours schistosomula extract antigen	42
Protein determination by Lowry's Method	43
Dialysis	44
Lyophilization	45
Human Sera	45
Purification of anti-Schistosoma Np-40 worm antigen antibodies by affinity chromatography	46
Purification of human IgG immunoglobulin by protein-G affinity column	48

Purification of human IgG3 subclass by protein-A affinity column	50
Purification of IgG1 and IgG4 subclasses specific to <u>Schistosoma mansoni</u> adult worm Np-40 extract ..	51
Enzyme-Linked immunosorbant Assay (ELISA)	53
Use of ELISA for the detection of IgM, total IgG and IgG subclasses specific to Np-40 extract of <u>Schistosoma mansoni</u> adult worms	56
Detection of the purity of IgG1 and IgG4 specific to Np-40 extract of <u>S. mansoni</u> adult worms by ELISA	57
Use of ELISA for determination of specificity of antibodies specific to Np-40 extract for adult worms and other schistosome antigen	58
Sodium dedecyl sulphate polyacrylamide gel Electrophoresis (SDS-PAGE)	59
Silver stain for Detecting protein in SDA-PAGE	63
Electrophoretic transfer of proteins from SDS-PAGE to nitrocellulose sheets (Immunoblotting)	65
Immunodetection of antigens on nitrocellulose strips reagents	65
Preparation of 3 hour schistosomula	67
Antibody dependent complemtn-mediated cytotoxicity "ADCC"	69
Indirect Immunofluorescence assay (IIF)	71
Statistical analysis	74
 - RESULTS	 76
- DISCUSSION	138
- SUMMARY	148
- REFERENCES	151
- ARABIC SUMMARY	

ABBREVIATIONS

Ab	Antibody.
ADCC	Antibody dependent complement mediate cytotoxicity.
Ag	Antigen.
BSA	Bovine serum albumin.
CHS	Chronic human serum.
CNBr-4B	Cyanogen bromide activated sepharose-4B.
ELISA	Enzyme-Linked immunosorbent assay.
Ig	Immunoglobulin.
IIF	Indirect immunofluorescence.
Mabs	Monoclonal antibodies.
Mr	Molecular weight.
Np ₋₄₀ AWA	Np ₋₄₀ adult worm antigen extract.
O.D.	Optical density.
PBS	Phosphate buffer saline.
PBS-T	Phosphate buffer saline-Tween20.
PMSF	Para-methyl sulfonide fluoride.
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis.
SEA	Soluble egg antigen.
<u>S. m</u>	<u>Schistosoma mansoni</u> .
SWAP	Soluble worm antigen preparation.

AIM OF THE WORK

AIM OF THE WORK

This protocol is intended to purify anti-adult worm Np-40 extract antibodies, their specific IgG and its subclasses from sera of S. mansoni chronic human infections. Specific IgG and its subclasses will be tested for purity, specificity for adult worm membrane antigen, recognition of epitopes on the surfaces of schistosomula, eggs and adult worms and ability to mediate in vitro complement-dependent killing of schistosomula.

INTRODUCTION

INTRODUCTION

I. Schistosomiasis

Schistosomiasis is a world-wide health problem affecting more than 200 million people in many countries (**Stockard, 1978**) and is considered the second most prevalent disease in the world next to malaria.

This disease was first recognized by the Egyptians 4000 years ago (**Ruffer, 1910**) and currently in Egypt schistosomiasis diminishing millions of people's productivity and exerting significant socio-economic impact. Moreover, this disease is increasing in both prevalence and distribution as man continues to produce an ecology suitable for disease transmission (**Jordan, 1972**).

Theodore Bilharz (1851 - 1853) in Cairo discovered the **Schistosome haematobium** worm secreting eggs with terminal spine in the urine of patients and provided the first description of many of the pathological sequelae of acute infection, whereas, a case from the Caribbean with schistosomiasis had many eggs with Lateral spine in the feces but not found in the urine (**Katz, et al., 1989**).

Sambson (1907) differentiated the two *Schistosoma* on the basis of morphology and origin of the eggs in the stool and urine.

The new organism, **Schistosoma mansoni**, was named by Sambson in tribute to Manson (**Sambson, 1907**).

In Egypt, nearly half of the population are infected with **Schistosoma mansoni** and /or **Schistosoma haematobium** (**Abdel-Wahab, et al., 1980**). Twelve to fifteen millions are infected with **S. mansoni**, and its prevalence is increasing in certain areas of Egypt's Nile delta (**El-Alamy and Cline, 1979; Abdel-Wahab, et al., 1979**), and are also spreading to areas where it was not previously recorded (**Ayad, 1974**).

Shistosomiasis mansoni is believed to be a causative factor in anemia, nutritional deficiencies and Liver diseases in Egypt. (**Abdel-Wahab, et al., 1980**).

In man, Schistosomiasis is caused by the three main trematode species belonging to the genus **Schistosoma**: **S. haematobium**, **S. Mansoni** and **S. japonicum** and their snail intermediate hosts belong to the genus **Bulinus**, **Biomphalaria** and **Onchomelania**, respectively. **S. mansoni** is found in Africa, Asia, South America and the Caribbean. **S. haematobium** is found in Africa, and the Near and Middle East. **S. japonicum** is located in the Far East (**Manson-Bahr, 1987**).

Human or animal excreta containing schistosome eggs contaminate water harbouring snail intermediate hosts. The