

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON DC 20523

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MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. _____

6. 390

PR# 3

Attachment

PN-100A-509

PROGRESS REPORT NO. 3

IDENTIFICATION OF SURFACE ANTIGENS AND PRODUCTION OF
MONOCLONAL ANTIBODIES IN SCHISTOSOMA JAPONICUM

6.39⁰

A RESEARCH PROJECT

USAID/PSTC PROGRAM

GRANT NO. 936-5542-G-00-6076-00

SUBMITTED BY

PRASERT SOBHON, PH.D.

PROJECT LEADER

ANATOMY DEPARTMENT

FACULTY OF SCIENCE, MAHIDOL UNIVERSITY

PROJECT PROFILE

COUNTRY	Thailand
GRANT NO.	936-5542-G-00-6076-00
PROGRAM	Program on Science and Technology Cooperation
PROJECT TITLE	Identification of Surface Antigen and Production of Monoclonal Antibodies in <u>Schistosoma japonicum</u>
PROJECT LEADER	Dr. Prasert Sobhon
ORGANIZATION	Faculty of Science, Mahidol University, Bangkok 10400, Thailand.
CO-INVESTIGATORS	Dr. Suchart Upatham Dr. Thanit Kusamran Dr. Vithoon Vaiyanant
PROJECT CONSULTANTS	-
AUTHORIZED OFFICER	Dr. Pairote Prempree, Dean, Faculty of Science, Mahidol University
TOTAL PROJECT BUDGET	US\$150,000.-
PROJECT DURATION	3 years
REPORTING PERIOD	August 1987 - March, 1988
BUDGET ALLOCATION FOR THIS PERIOD	465,439 Bahts

C O N T E N T

1. BACKGROUND

The grant was awarded on August 11, 1986. During the one a half year period we have finished the identification and characterization of major antigenic proteins from the surface of adult S. japonicum by using immunoprecipitation method. These proteins were also compared with another species of oriental schistosome, ie., S. mekongi, and S. mansoni. In this report we would like to present the work on identification and characterization of antigenic surface proteins and lipids in adult of S. japonicum and other schistosome species using immunoblotting. Since this technique is more convenient to perform, we intend to use it as the principal method to detect specific monoclonal antibodies (MAB) to be raised against surface antigens. Therefore, first we have to verify that the antigens (especially proteins) detected by this method do not differ greatly from that obtained from immunoprecipitation technique, which is more difficult to perform and not convenient for use in the screening of MAB(s).

2. OBJECTIVES OF THE PROJECT

- 2.1 To identify and characterize antigens on the surface of the tegument of cercariae, schistosomula and adult Schistosoma japonicum (Chinese & Philippine), and to study their cross-reactions with surface antigens of other schistosome species.
- 2.2 To produce monoclonal antibodies (MAB) against parasite surface antigens identify MAB that can kill schistosomula, and those that can react with specific surface antigens, which could be used for immuno diagnosis.

3. MATERIALS & METHODS

In this period we have performed 2 major experiments as follows :

3.1 Identification and comparison of immunogenic surface protein antigens of adult *S. japonicum*, *S. mekongi* and *S. mansoni* by immunoblotting

In this experiment, the surface antigens of parasites were obtained as follows : the parasites' tegument were extracted by freeze-thawing on dried ice in tris-buffer for 20 cycles, and the shed pieces of tegument in the supernatant were collected after centrifugation of the mixture at 100 x g for 5 min. The supernatant was solubilized in the SDS-PAGE sample buffer and applied onto 12.5% SDS-PAGE gels. Then, electrophoresis was performed at 100 volts for 4 hrs, and the proteins on gel were blotted onto nitrocellulose (NC) paper using blotting apparatus with setting at 0.5 mA for 1 hr. Antigenic proteins on the nitrocellulose paper were detected by incubating NC strips in infected mouse serum and followed by rabbit anti-mouse Ig labelled with horseradish peroxidase (HRP). The immunogenic proteins that bind with anti-mouse Ig-HRP were visualized by incubating the nitrocellulose papers in 3,3-diaminobenzidine and H_2O_2 .

3.2 Identification of lipids on the surface of *S. japonicum*

The presence of lipids as membrane surface components have been reported in various species of parasitic helminths, including *S. mansoni*. Some of the lipids have been demonstrated to be immunogenic in both animals or human infected by the parasites.

In this experiment, living adult *S. japonicum* (Philippines) were ^{125}I -labelled by a similar procedure as used in the protein labelling according to immunoprecipitation method. Freeze-thawed tegument of the parasite was prepared and lyophilized into dry powder and lipid components were extracted by hexane-isopropanol. The

solvent could reproducibly recover the majority of the radioactively labelled compounds, whereas the precipitate (presumably proteins) always represented less than 20%, suggesting that lipids were major components of the *S. japonicum* tegument. The lipid fraction was further extracted in an aqueous system to separate polar lipids (glycolipids/lipoproteins) from non-polar lipids; and it was found that there are glycolipids about 3-4 times higher than non-glycolipids as determined from their radioactivities. The radioactively labelled lipids were observed to bind tightly to the Eppendorf plastic tubes leading to marked loss during the extraction. However, $10^5 - 10^6$ cpm of the ^{125}I -glycolipids were usually obtained following the extraction. Analysis of labelled lipids were performed on thin layer chromatography, and revealed by autoradiography in comparison with standard lipids.

4. RESULTS & DISCUSSION

4.1 Identification and comparison of immunogenic surface protein antigens of adult *S. japonicum* and other schistosomes by immunoblotting (Table 1, Figure 1)

It was found that in all species most bands that occur in immunoblotting correspond to those present in immunoprecipitation. However, there are generally fewer bands (up to 5 or 6) in immunoblotting than in immunoprecipitation, and the loss is greatest among bands with MW from 120,000 upwards. There are also slight shifts in MW of some bands in comparison to immunoprecipitation. However, after taking this into account, it appears that there are three regions with strong cross-reactivities among various species. The first and most intense are two bands at MW between 80,000 to 86,000 and at 68,000, the second is at MW 29,500 to 31,500, and the third at MW 37,000 to 38,500. Immunoprecipitation experiment using *S. mansoni* antigen (MA) as a starting material shows that there is also consistent and strong

cross reactivities among bands 29,500 to 26,500, whereas the cross reactivities at MW 68,000 and 80,000 to 85,000 are much less pronounced (Table 1, Figure 1). It should also be noted that bands 68,000 and 86,000 of S. japonicum are not intense in immunoprecipitation but appear very intense in immunoblotting. It is probable that these bands are "hidden" or not so rich in tyrosine, and therefore not easily accessible to ^{125}I labelling, while antibodies against them are well generated. It is, therefore, possible that these proteins are major surface proteins in adult oriental schistosome that could be used for detecting MAB.

4.2 Identification of antigenic surface lipids

Analysis on thin-layer chromatography (TLC, n-butanol : acetic acid : water = 3:1:1) and autoradiography revealed at least 3 main components are present in the glycolipid preparation. Identification of these lipids are under investigation.

Antigenic property of the glycolipids was also investigated by immunoprecipitation using mouse immune serum and protein A-Sepharose. Only 0.4% of the radioactivity (200 cpm from 5.3×10^4 cpm) was found in the immunoprecipitate. The proportion was rather small and it was uncertain whether this value significantly represented the surface glycolipid antigens of the S. japonicum. Additional experiments are being conducted to confirm the results by using increasing amount of the sample and glass tubes (to replace plastic tubes) to increase the recovery yield during the extraction.

Furthermore, an alternative approach will be attempted to confirm the above results by using the procedure which has recently been reported for studying glycolipid antigens of Leishmania major (Rosen, et al., 1988, Molecular and Biochemical Parasitology 27 : 93-100). Both glycolipids and non-glycolipids of S. japonicum will be separated on TLC and transferred by blotting to a nitrocellulose

membrane on which the antigens (if present) will be revealed by conjugating with homologous immune sera from either mouse or human. The immune complex will be subsequently detected by ^{125}I -protein A, and autoradiography performed. It will also be of interest to examine cross immunoreaction of the lipid antigens among various strains and species of schistosomes as well as the possible detection of circulating glycolipid antigens and their antisera.

5. CONCLUSION & REMARKS

We have demonstrated that the surface proteins at MW 86,000; 80,000 and 68,000 are major antigenic proteins of *S. japonicum* as shown by immunoblotting, and that these proteins are quite specific to oriental schistosomes. If MAB can be developed against them, they could probably be used for immunodiagnosis by the adaptation of immunoblotting technique.

6. WORKPLAN FOR THE NEXT PERIOD

- 6.1 Attempt will be made to identify and characterize proteins that may be released into the medium and hosts' sera from the parasites, and compare with the surface proteins as obtained from immunoprecipitation and immunoblotting.
- 6.2 The screening for positive MAB clones against surface proteins that have been identified by using immunoblotting will be performed.
- 6.3 The identification of MAB clones that bind to the surface of parasites by immunofluorescence technique will be also screened.
- 6.4 Further study on immunogenic lipids will also be performed.

TABLE 1 IMMUNOBLOTTING PATTERNS OF PROTEINS DERIVED FROM FREEZE-THAWED ADULT TEGUMENT OF S. MANSONI (MA), S. JAPONICUM (CHINESE-CH), S. JAPONICUM (PHILIPPINE-PH) AND S. MEKONGI (ME) AGAINST MOUSE ANTISERA AGAINST S. MANSONI (ISMA), S. JAPONICUM (CHINESE-ISCH), S. JAPONICUM (PHILIPPINE-ISPH) AND S. MEKONGI (ISME); AND CON-A STAINING PATTERN OF THESE PROTEINS

MA* + ISMA	CH* + ISCH	ME* + ISME	ISMA			ISCH & ISPH			ISME		
			MA	CH&PH	ME	MA	CH&PH	ME	MA	CH&PH	ME
205	205		+		+			+			+
	180	185	+	+	+			+			+
160	158	155			+			+			+
	128							+			
	114							+			
105			+	+		+				+	+
		94		+	+			+	+		++(*)
	86	85			+			++(*)	++		++(*)
80			++(*)			+				+	
	68	68	++		+	+		++(*)	++	+	++(*)
64	62	64									
58								+			
		54									
42.5	45	43		+				+			
37	38	38.5	++		++	+	+		++	+	++(*)
34	34	35	+	+		+		+			
29.5	30	31.5	+	+	++			+	++		++(*)
26.5	26	26	++			+		+		+	
22.5	23							+			
19	19										
<15	<15	<15									

"+" Signs indicates relative intensity after incubating NC strips in rabbit anti-mouse Ig linked to HRP, and staining with DAB + H₂O₂.

Signs indicates whether the bands also show up when the NC strips are stained with Con A.

MA* CH* ME* are immunoprecipitation patterns of parasites' ¹²⁵I labelled surface antigens with corresponding immune sera. (See Progress Report #2).

FIGURE 1 IMMUNOBLOTTINGS OF ANTIGENIC PROTEINS FROM THE TEGUMENT OF S. MANSONI (MA), S. JAPONICUM (CHINESE & PHILIPPINE) (CH&PH), S. MEKONGI (ME). THE TEGUMENTS WERE EXTRACTED FROM THE PARASITES BY FREEZE-THAWING, AND SOLUBILIZED IN THE SAMPLE BUFFER. ELECTROPHORESIS WAS PERFORMED ON 12.5% SDS-PAGE, AND PROTEIN BANDS TRANSFERRED ONTO NITROCELLULOSE (NC) PAPER. THE NC STRIPS WERE OVERLAID WITH MOUSE IMMUNE SERA ISMA, ISCH, ISPH AND ISME, AND IMMUNOGENIC BANDS WERE DETECTED BY RABBIT ANTI-MOUSE IgG LABELLED WITH HORSE-RADISH PEROXIDASE (HRP). THE COLOR WAS DEVELOPED BY INCUBATING WITH DAB + H₂O₂.

Experiment 1 :	A. Ag(s) + ISMA	B. Ag(s) + ISCH
(blotting time-1 hr)	C. Ag(s) + ISPH	D. Ag(s) + ISME
Experiment 2 :	E. Ag(s) + ISMA	F. Ag(s) + ISCH
(blotting time-1½ hr)	G. Ag(s) + ISPH	H. Ag(s) + ISME

Figure 1

