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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 10/26/87

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 #2 rec'd 10/21/87

Attachment

2 cy

6.390

PROGRESS REPORT NO. 2

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

A RESEARCH PROJECT

USAID/PSTC PROGRAM

Grant No. 936-5542-G-00-6076-00

Submitted by

PRASERT SOBHON, PH.D.

PROJECT LEADER

DEPARTMENT OF ANATOMY

FACULTY OF SCIENCE, MAHIDOL UNIVERSITY

Rec'd in S OCT 26 1987

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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 *Schistosoma japonicum*
Project Leader :	Dr. Prasert Sobhon
Organization :	Faculty of Science, Mahidol University, Bangkok 10400, Thailand.
Co-investigators :	Dr. Suchart Upatham Dr. Thanit Kusamran Dr. Vithoon Viyanant
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 :	February 1987 - July 1987
Budget Allocation for this Period :	574,943 Bahts

IDENTIFICATION OF SURFACE ANTIGENS AND PRODUCTION OF MONOCLONAL ANTIBODIES IN SCHISTOSOMA JAPONICUM (CHINESE & PHILIPPINE)

PRASERT SOBHON, WANNANA PANUWATSUK, CHINNAVAT THARASUB, SUCHART UPATHAM, THANIT KUSAMRAN AND VITHOON VIYANANT

Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

ABSTRACT

The patterns of ^{125}I-labelled surface proteins and immunoprecipitations by mouse antisera against S. japonicum (Chinese) (ISCH), against S. japonicum (Philippine) (ISPH), against S. mansoni (ISMA) and human immune serum against S. japonicum (Philippine) (IHPH) are identical for adults of both strains of S. japonicum. The ^{125}I-labelled surface proteins consist of 18 bands, with the heaviest labelling at bands 180,000; 158,000; 128,000 and 15,000. Immunoprecipitations by ISCH, ISPH, IHPH result in similar patterns. The most intense band that stands out in contrast to others is 128,000, which is not precipitated by ISMA, mouse antiserum against S. mekongi (ISME), nor mouse antiserum against S. hematobium (ISHE); thus it is the most unique and specific antigen detected only in S. japonicum. Other bands with moderate precipitation against ISCH, ISPH and IHPH are 205,000: 180,000; 158,000; 45,000; 38,000; 34,000; 30,000; 26,000 and 23,000. It should be noted that mouse and human antisera produce identical patterns and that these bands show slight to moderate cross-reactions with ISMA. Band 15,000 is probably non-specifically precipitated, since it is always intense against all antisera as well as non-immune serum. In addition, it was found that band 86,000 is specifically and heavily precipitated against ISME and ISHE. The rest shows similar and light cross-reactions to ISME and ISHE as against ISMA. In conclusion, adults of S. japonicum (Chinese & Philippine) show a major and non-cross reacting surface antigens against ISMA, ISME and ISHE at 128,000 and 158,000, and specifically cross-reacting antigen against ISME and ISHE at 86,000. The rest of the surface proteins show light to moderate cross-reactions against ISMA, ISME and ISHE.

INTRODUCTION & OBJECTIVES

Human schistosomiasis is still a serious public health problem in China, the Philippines, Indonesia and Indo-Chinese states that include parts of Laos, Cambodia and Vietnam[1-4]. A few sporadic cases of human schistosomiasis were reported in the northern, north-eastern parts of Thailand bordering Laos and Cambodia and in the southern part of Thailand[4], Pahang and Perak states of Malaysia[5-7]. In Indonesia, schistosomiasis is endemic in the region around Lake Lindoe in central Sulawesi[8-9]. Two predominant species of schistosomes were found to be responsible for schistosomiasis in the Orient, namely, Schistosoma japonicum and Schistosoma mekongi[3-4]. The former consists of various geographic strains; of which the Chinese and Philippine strains are medically the most important because of their wide spread. On the other hand, S. mekongi is responsible for schistosomiasis in the Lower Mekong Basin area that encompasses parts of Laos, Cambodia and north-eastern Thailand[4].

One approach adopted as one of the main strategies for control of schistosomiasis is laboratory studies that will ultimately lead to the production of vaccines, that can be conveniently administered to the population at risk. The rationale for the development of vaccines is based on the finding that when human hosts received primary infection, both humoral and cell-mediated immunities are induced. These concomitant immunities protect hosts against reinfecting schistosomulae, though the adult worms from the primary infection have evolved an evasion mechanism that make them resistant to the hosts' immunities[10]. It has now been established both in vivo and in vitro that young schistosomulae are only susceptible to immune effector mechanisms within the first few days of their transformation from cercariae[11,12]. The primary goal of using vaccines is, therefore, to induce immune mechanism in hosts that can kill the schistosomulae at this stage. It is amply demonstrated that the immune attack occurs on the surface of young parasites; and reciprocally, older parasites can evade host immune attacks through the modification of their outer membrane. Hence, biochemical and immunochemical characterization of parasite tegument, especially that of the outer membrane, is the key to the understanding of interaction between the host immune system and the parasites, and the latter's evasion mechanism. In addition, the identification and characterization of major surface antigens that can elicit concomitant immunity in hosts is essential if some of them are to be selected for use in the new immunodiagnostic methods as well as for an eventual production of the vaccines. Therefore, in this project our major objectives are as follows :
1. To identify and characterize antigens on the surface of the tegument of S.japonicum (Chinese & Philippine), and to study cross-reactions with surface antigens of other schistosome species, in order to define major antigens that can illicit concomittant immunity in hosts ; (2) To produce monoclonal antibodies (MAB) against these surface antigens, so that MAB can be used to detect major surface antigens that can confer protective immunity. Consequently, these antigens and MAB can be used for improvement of immunodiagnostic methods and possibly future production of vaccines.

In this report the first part of the results concerning the identification and characterization of major surface antigens of adult S. japonicum (Chinese & Philippine) are presented.

MATERIALS AND METHODS

Collection of adult schistosomes. S. japonicum (Chinese & Philippine) cycles are maintained in Oncomelania hupensis hupensis and Oncomelania hupensis quadrasi under laboratory conditions. Adult worms were obtained from Swiss albino mice, infected 8-week previously with cercariae, by portal perfusion with 0.15 M sodium chloride and 0.05 M sodium citrate. The worms were washed 5 times with Minimum Essential Medium (MEM) (GIBCO) before used.

Collection of antisera. Infected mouse antisera were obtained from Swiss albino mice, each infected with 30 cercariae for 8 weeks, by heart puncture. Animals were bled and the blood was allowed to clot at 25° C for 3 hours. The clot was removed and the antisera were centrifuged at 1000 x g for 5 minutes to obtain clear supernatant. Normal mouse serum was obtained in a similar manner from uninfected mice, and the normal human serum was obtained from volunteers. In addition, human antiserum against S. japonicum (Philippine) was kindly provided by Professor F.G. Garcia of the Institute of Public Health, The University of Philippine, Manila, The Philippines.

Surface antigen labelling with ^{125}I. Adult male S. japonicum were radiolabelled by ^{125}I using Iodogen method of Fraker & Speck, (1987)[13] by using 100 uCi $Na^{125}I$, 100 ug Iodogen (Pierce Chemical Company) per 100 adult male parasites. The labelling time was 5 minutes and this was stopped and chased with 5 mM KI. Labelled parasites were rapidly frozen and thawed in 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 10 mM EDTA and 1 mM PMSF (TNEP buffer) in cycles using dry-ice for 10 times.

Immunoprecipitation of ^{125}I surface antigens by immune sera. Immunoprecipitations were set up using labelled surface antigens from freeze-thawed fractions of tegument at 100 ul (10^6 ct/min) per aliquot, and these were clarified by incubation with 10 ul of normal serum. After 10 minutes the complexes were precipitated by the addition of 100 ul of 10% protein-A Sepharose and centrifuged at 2000 x g for 10 minutes at 4°C. Supernatants were incubated with specie-specific immune sera as well as immune sera against other schistosoma for 30 minutes at 37°C, and kept further for overnight (18 hrs) at 4°C. The antigen-antibody complexes were then precipitated with protein-A sepharose. The immunoprecipitates were centrifuged at 2000 x g for 10 minutes at 4°C to get a pellet and then washed 3 times in phosphate buffer saline pH 7.4. Each of the pellets were then treated with 50 ul of sample buffer (62.5 mM Tris-HCl, pH 6.8; 2% SDS: 5% β-Mercaptoethanol and 10% Glycerol) and boiled for 2 minutes to complete the dissolution.

Electrophoresis and autoradiography SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1971)[14]. Electrophoresis was performed on 12.5% gels. The voltage was set at 100 volts and gel run for 4½ hrs. After drying, the gels were exposed to X-ray film (Kodak XO mat RP film) using intensifying screens. Autoradiographs were developed in Kodak GBX using developing time for 2 minutes.

EM autoradiography (EMARG) of labelled worms. In order to monitor the sites of ^{125}I labelling EMARG was performed. The worms were washed after ^{125}I labelling, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2 for 18 hrs. at 4°C. After rinsing 3 times in cacodylate buffer, the worms were postfixed in 1% OsO_4 in cacodylate buffer for 2 hrs and washed 3 times with cacodylate buffer. Following washings, worms were dehydrated and embedded in Araldite 502. About 300-500 Å thin sections were cut with Porter-Plum MT-2 ultramicrotome and coated with Ilford L-4 emulsion maintained at 45°C and diluted 1:1 (V/V) with H_2O. Autoradiographs were exposed, developed in undiluted Dektol developer (Eastman Kodak Company) for 2 mins., fixed for 10 mins., washed in water, dried and stained with lead citrate and uranyl acetate.

RESULTS AND DISCUSSION

Identification and Comparison of Immunogenic Surface Proteins and Glycoproteins of Adult S. mansoni, S. japonicum (Chinese & Philippine) by Immunoprecipitation (Tables 1 to 3; Plates 1-3)

Immunoprecipitation of surface antigens from S. mansoni (Table 1 ; Plate 2 A,B)

For comparison with oriental schistosomes, we have done an experiment on adult S. mansoni under similar conditions. Our results indicate that the ^{125}I-labelled surface proteins of adult S. mansoni consists of 15 bands, the most intense are bands with M.W. 26,500; 29,500 and < 15,000, and the moderately intense ones are 64,000; 58,000 and 42,500. Fourteen bands are precipitated by mouse immune serum against S. mansoni (ISMA), with the heaviest precipitation at 15,000, 26,500 and 29,500. Mouse immune sera against S. japonicum (Chinese & Philippine - ISCH & ISPH), against S. mekongi (ISME), and against S. haematobium (ISHE) all show cross-reactions with S. mansoni antigens (MA), with heaviest precipitations at 15,000, 26,500 and 29,500, respectively. However, we believe that band 15,000 is non-specific, since it can be precipitated by all immune sera, as well as non-immune serum. This also occurs in other parasites, and it is possible that this band represents small molecular weight, fast moving lipoproteins or glycolipids of the surface membrane.

The surface antigens of S. mansoni have been characterized previously by many groups of investigators, with slightly varying results. By using radio-labelling with ^{125}I or ^{3}H and one-dimension SDS gel electrophoresis, differences in the number and exact molecular weights have been reported; however, most investigators generally agreed that there are about 7 to 15 bands of surface proteins and glycoproteins, ranging in molecular weights from 12,000 to 200,000 daltons. Most labelled proteins and glycoproteins could be immunoprecipitated with antisera from patients and infected animals; the reported molecular weights of major precipitated protein and glycoprotein antigens vary considerably[15-25]. Taylor et al.[19], utilizing iodosulphanilic acid to label schistosomulae, could precipitate polypeptides with MW 185,000; 68,000 and 24,000 daltons with infected human sera. Dissous et al.[20], on the other hand, could immunoprecipitate polypeptides with MW 100,000; 40,000; 37,000 and 32,000 and 20,000 - 25,000 with immuned rat and human sera, following the ^{125}I labelling of schistosomula surface antigens by lactoperoxidase method. Using similar technique, Simpson et al[24], showed that there are seven major proteins with MW 94,000, 68,000, 45,000, 40,000 to 32,000, 22,000 and 16,000 on the surface of 3-hour schistosomulae. On the other hand, Samuelson & Caulfield[23] using galactose oxidase/Na B^3H_4 revealed 12 bands of major surface glycoproteins and 7 glycolipids on newly transformed schistosomulae. By contrast, Strand et al.[22] could immunoprecipitate up to 15 bands of glycoproteins (MW 200,000 to 12,000) isolated from the surfaces of cercariae and adult parasites by ConA-affinity chromatography. Indeed, the number of immunoprecipitable surface proteins and glycoproteins in schistosomulae can be as high as 35 bands if resolved by two-dimensional gel electrophoresis 22 In general, it was agreed that, despite the difference in morphology of various developmental stages from cercaria to adult, it was found that the surface proteins of cercariae and various stages schistosomulae and even adult also showed marked similarity[15,23,2629].

Furthermore, despites the variations in the n umber of bands and their molecular weithts, recent studies revealed that schistosomulae, lung-stage and adult worms share some common surface antigens which may become "hidden" in the latter[28,29]. Dissous & Capron (1983)[28] demonstrated a common antigenic determinant shared by surface molecules with MW 38,000 daltons on newly transformed schistosomulae and 115,000 daltons on adult parasites. Payares et al. (1985)[29] also showed that schistosomulae, lung stage and adult worms bear prominent common surface antigens with MW 32,000 to 38,000 and 20,000 daltons, whereas an antigen with MW 25,000 daltons was found only in lung stage and adult worms. Furthermore, it was shown in these studies that antisera against the purified membrane of adult parasites and monoclonal antibodies raised against adult antigens can readily kill schistosomulae in vitro. These evidence strongly support the concept of "concomitant immunity", and demonstrated the possibility that certain surface antigens may be selected as potential candidates for the ultimate production of vaccines.

Immunoprecipitation of surface antigens from adult S. japonicum (Chinese & Philippine) (Table 2 & 3; Plates 1,2 C, D & 3 A, B).

The results obtained from EMARG indicate that over 95% of positive silver grains occurs on the membrane covering ridges or pits of the parasites' tegument (Pl. 1); therefore, it is save to assume that the labelling by ^{125}I occurs primarily on the proteins associated with the surface membrane of the parasites' tegument. The patterns of ^{125}I-labelled surface proteins and immunoprecipitations by ISCH, ISPH, ISMA and human immune serum against S. japonicum (Philippine) (IHPH) are identical for both strains. The ^{125}I-labelled surface proteins consist of 18 bands, with the heaviest labelling at bands 180,000; 158,000; 128,000 and 15,000. Immunoprecipitations by ISCH, ISPH, IHPH result in similar patterns. The most intense band that stands out in contrast to others is 128,000 which is not precipitated by ISMA, ISME, nor ISHE; thus it is the most unique and specific antigen detected only in S. japonicum. Other bands with moderate precipitation against ISCH, ISPH and IHPH are 205,000; 180,000; 158,000; 45,000; 38,000; 34,000; 30,000; 26,000 and 23,000. It should be noted that

mouse and human antisera produce identical patterns and that these bands show slight to moderate cross-reactions with ISMA. Like S. mansoni, band 15,000 is probably non-specifically precipitated, since it is always intense against all antisera as well as non-immune serum. In addition, it was found that band 86,000 is specifically and heavily precipitated against ISME and ISHE. The rest show similar and light cross-reactions to ISME and ISHE as against ISMA.

In conclusion, adults of S. japonicum (Chinese & Philippine) show major and non-cross reacting surface antigens against ISME, ISME and ISHE at 128,000 and 158,000 and specifically cross-reacting antigen against ISME and ISHE at 86,000. The rest of the surface proteins show light to moderate cross-reactions against ISMA, ISME and ISHE.

TABLE 1 Immunoprecipitations of ^{125}I-labelled surface proteins of adult male S. mansoni by mouse antisera against S. mansoni (ISMA), against S. japonicum (Chinese & Philippine - ISCH & ISPH), against S. mekongi (ISME) and against S. hematobium (ISHE), and comparison to the results on adult S. mansoni as reported by Shah and Ramasamy (1982)[15] and Hayunga (1979 a, b)[16,17].

(Refer to Plate 2 A & B)

Immunoprecipitations of adult S.mansoni ^{125}I-labelled Surface proteins as reported by		^{125}I labelled surface proteins of S. mansoni	Immunoprecipitations of adult male S. mansoni ^{125}I-labelled surface proteins by				
Shah & Ramasamy (1982)* (MW X 10^{-3})	Hayunga et al 1979 (ab)** (MW X 10^{-3})	(MW X 10^{-3})	ISMA	ISCH	ISPH	ISME	ISHE
			(ME X 10^{-3})				
-	-	> 205 (+)	++	+	+	+	+
>150	-	160 (+)	++	+	+	+	+
102	100	105 (+)	++	+	+	+	+
78	78	80 (+)	+	-	-	-	-
-	68	64 (++)	++	+	+	+	+
59	60	58 (++)	++	-	-	-	-
-	-	50 (+)	-	-	-	-	-
45	43	12.5(++)	++	+	+	+	+
-	36	37 (+)	+	-	-	-	-
35	-	34 (+)	+	+	+	+	+
-	30	29.5(++)	++	++	++	++	++
-	26	26.5(+++)	+++	++	++	++	++
22	20	22.5	+	+	+	+	+
-	-	19 (++)	++	+	+	+	+
-	12	< 15 (+++)	+++	+++	+++	+++	+++
		(15 bands)					

"+" sign indicates relative intensity of the protein bands; "+" = light; "++" = moderate; "+++" = intense

* Shah, J. & Ramasamy, K. (1982) Int. J. Parasitol. 12, 451-461.
Hayunga, E.G., Murrell, K.D., Taylor, D.W. & Vannier, W.E. (1979 a) J. Parasitol., 65, 488-496.

** Hayunga, E.G., Murrell, K.D., Taylor, D.W. & Vannier, W.E. (1979 b) J. Parasitol., 65, 497-506.

TABLE 2 Immunoprecipitations of ^{125}I labelled surface proteins of adult male S. japonicum (Chinese) by mouse antisera against S. japonicum (Chinese & Philippine - ISCH & ISPH), human antisera against S. japonicum (Philippine - IHPH)*, mouse antisera against S. mekongi (ISME), S. mansoni (ISMA) and S. hematobium (ISHE). All samples were pre-absorbed with normal mouse or normal human sera.

(Refer to Plate 2 C & D)

^{125}I Labelled Surface Proteins (MW X 10^{-3})	Immunoprecipitated by ISCH,ISPH,IHPH (MW X 10^{-3})	Immunoprecipitated by ISME, ISHE (MW X 10^{-3})	Immunoprecipitated by ISMA (MW X 10^{-3})
205 (+)	++	+	+
180 (++)	++	+	+
158 (++)	++	-	-
128 (++)	+++	-	-
114 (+)	+	+	+
86 (+)	+	++	-
68 (+)	+	+	+
62 (+)	+	+	+
56 (+)	-	-	-
48 (+)	-	-	-
45 (+)	++	+	+
38 (+)	++	+	+
34 (+)	++	+	+
30 (+)	++	+	+
26 (+)	+	+	+
23 (+)	+	+	+
19 (+)	+	+	+
< 15 (+++)	+++	+++	+++
(18 bands)			

"+" sign indicates relative intensity ; "+" = light; "++" = moderate; "+++" = intense.

* IHPH - kindly supplied by Prof. E. Garcia, Institute of Pubiic Health, University of the Philippine System, Manila.

TABLE 3 Immunoprecipitations of ^{125}I labelled surface proteins of adult male *S. japonicum* (Philippine) by mouse antisera against *S. japonicum* (Chinese & Philippine - ISCH & ISPH), human antisera against *S. japonicum* (Philippine - IHPH), and mouse antisera against *S. mansoni* (ISMA). All samples were pre-absorbed with normal mouse or normal human sera.

(Refer to Plate 3 B)

^{125}I labelled Surface Proteins	Immunoprecipitated by ISPH, ISCH, IHPH	Immunoprecipitated by ISMA
(MW X 10^{-3})	(MW X 10^{-3})	(MW X 10^{-3})
205 (++)	++	++
180 (++)	++	++
158 (++)	+	-
128 (++)	+++	-
114 (+)	+	+
86 (+)	+	+
68 (+)	-	-
62 (+)	+	+
56 (+)	-	-
48 (+)	-	-
45 (+)	++	++
38 (+)	++	++
34 (+)	++	+
30 (+)	++	+
26 (+)	++	+
23 (+)	++	+
19 (+)	-	-
< 15 (+++)	+++	+++
(18 bands)		

REFERENCES

1. Hunter, G.W. III, Freyage, R.E., Richie, L.S., Fan, C., Yokogama, M. & Potts, D.E. Amer. J. Trop. Med. Hyg. (1952) 1:831-847

2. Santos, A.T. Southeast Asian J. Trop. Med. Public Health (1976) 7:133-136.

3. Harinasuth, C. Malacological Review, Suppl. 2 (1980) pp. 1-8.

4. Sornmani, S., Kitikoon, V., Thirachantra, S. & Harinasuta, C. Malacological Review, Suppl. 2 (1980) pp. 9-18.

5. Leong, S.H., Murugasu, R. & Chong, K.C. Proc. Tenth Malaysian-Singapore Congr. Med. (1975) 10:184.

6. Murugasu, R. & Dissanaike, A.S. Trans. Roy. Soc. Trop. Med. Hyg. (1973) 67 : 880.

7. Murugasu, R., Wang, F. & Dissanaike, A.S. Trans. Roy. Soc. Trop. Med. Hyg. (1978) 72:389.

8. Carney, W.P., Hadidjaja, P., Davis, G.M., Clarke, M.D., Djajasasmita, M. & Nalim, S. J. Parasitol. (1973) 59:210-211.

9. Carney, W.P., Marsi, S., Sudoma, M., Putrali, J. & Davis, G.M. Southeast Asian J. Trop. Med. Public. Health (1975) 6 (12):211-217.

10. Smithers, S.R. & Doenhoff, M.J. Immunology of Parasitic Infections. Ed. Cohen, S. & Warren, K.S. Blackwell Scientific Publication (1982) pp. 527-607.

11. Smithers, S.R. & Ramalho-Pinto, F.J. In : Immunity in Parasitic Diseases. Les Colloques de l'Institut National de la Sante et de la Researche Medicale. INSERM (1977) 72:39.

12. Sher, A., Smithers, S.R., Mackenzie, P. & Broomfield, K. Exp. Parasitol. (1977) 41:160.

13. Fraker, P.J. & Speck, J.C. Biochem. Biophys. Res. Comm. (1978) 80:849-857.

14. Laemmli, U.K. (1970) Nature (London) 277, 680.

15. Ramasamy, R. Int. J. Parasitol. (1979) 9:491-493

16. Hayunga, E.G., Murrell, K.D., Taylor, D.W. & Vannier, W.E. J. Parasitol. (1979a) 65:488-496.

17 Hayunga, E.G., Murrell, K.D., Taylor, D.W. & Vannier, W.E. J. Parasitol. (1979b) 65:497-506.

18. Snary, D., Smith, M.A. & Clegg, J.A. Eur. J. Immunol. (1980) 10:573-575.

19. Taylor, D.W., Hayunga, E.G. & Vannier, W.E. Mol. Biochem. Parasitol (1981)

20. Dissous, C., Dissous, C. & Capron, A. Mol. Biochem. Parasitol. (1981) 3: 215-225.

21. Dissous, C., Grzych, J.M. & Capron, A. J. Immunol. (1982) 129:2232-2234.

22. Strand, M., McMillan, A. & Pan, X.Q. Exp. Parasitol. (1982) 54:145-156.

23. Samuelson, J.C. & Caulfield, J.P. J. Cell Biol. (1982) 94:363-369.

24. Simpson, A.J.G., James, S.L. & Sher, A. Infection and Immunity (1983) 41:591-597.

25. Taylor, D.W. & Wells, P.Z. Parasitology (1984) 89:495-510.

26. Taveres, C.A.P., Cordiero, M.N., Motasantos, T.A. & Gazzinelli, G. Parasitology (1980) 80:95-104.

27. Shah, J. & Ramasamy, R. Int. J. Parasitol. (1982) 12:451-461.

28. Dissous, C. & Capron, A. FEBS Letters (1983) 162:355-359.

29. Payares, G., McLaren, D.J., Evans, W.H. & Smithers, S.R. Parasite Immunology (1985) 7:45-61.

บทคัดย่อ

จากการติดฉลากด้วย ^{125}I และการวิเคราะห์ด้วยกรรมวิธีอีเล็กโตรฟอรีซีสกับอิมมูโนพริซิปิเตชั่น ปรากฏว่าโปรตีนแอนติเจนแบบผิวของพยาธิตัวแก่จาก S. japonicum (Chinese & Philippine) มีความคล้ายคลึงกันมาก โปรตีนบนผิวที่ติดฉลากด้วย ^{125}I มี 18 แถบ และมีความเข้มสุดที่ M.Wt. 180,000; 158,000; 128,000 และ 15,000 โปรตีนบนผิวที่ตกตะกอนด้วยแอนติบอดี้จากหนูต่อ S. japonicum (Chinese)-ISCH, ต่อ S. japonicum (Philippine)-ISPH และจากคนไข้ที่ติดพยาธิ S. japonicum (Philippine)-IHPH มีลักษณะคล้ายคลึงกันแถบที่เด่นที่สุด คือแถบ 128,000 ซึ่งไม่ตกตะกอนด้วยแอนติบอดี้จากหนูที่ติดพยาธิ S. mansoni (ISMA) S. mekongi (ISME) และ S. haematobium (ISHE) ดังนั้นแถบนี้จึงเป็นแถบเฉพาะของ S. japonicum ส่วนแถบอื่น ๆ ที่ตกตะกอนด้วย ISCH, ISPH และ IHPH ได้แก่ 205,000; 180,000; 158,000; 45,000; 38,000; 34,000; 30,000; 26,000 และ 23,000 แถบเหล่านี้ยังตกตะกอนได้พอประมาณ ถึงได้น้อยกับ ISMA ส่วนแถบ 15,000 คงจะเป็นไลโปโปรตีน หรือไกลโคลิปิดที่มีโมเลกุลขนาดเล็ก และมีอยู่บนผิวพยาธิทุกชนิด เนื่องจากมันสามารถตกตะกอนได้ทั่วไปกับแอนติบอดี้ทุกอย่างรวมทั้งที่เป็นเซรุ่มจากกลุ่มควบคุมด้วย

PLATE 1 Electron microscopic autoradiography (EM-ARG) to detect the ^{125}I-labelling sites on the tegument of S. japonicum (Chinese & Philippine) following iodination by iodogen method.

A&B) EM-ARG of the tegument of an adult male S. japoncium (Chinese). Notice that most silver grains (arrow heads) lie either just outside or on the membrane of pits (Pt) and ridges (Ri). X 20,000.

PLATE 2 Immunoprecipitation patterns of ^{125}I-labelled surface proteins from adult male S. mansoni (MA), S. japonicum (Chinese - CH) by mouse immune sera against S. mansoni (ISMA), S. japonicum (Chinese & Philippine - ISCH & ISPH), S hematobium (ISHE), S. mekongi (ISME), and human immune sera against S. japonicum (Philippine - IHPH). The gels used in all cases were 12.5% SDS-PAGE, and all samples were preabsorbed with non-immune mouse serum (NMS).

A & B) duplicate experiments on S. mansoni

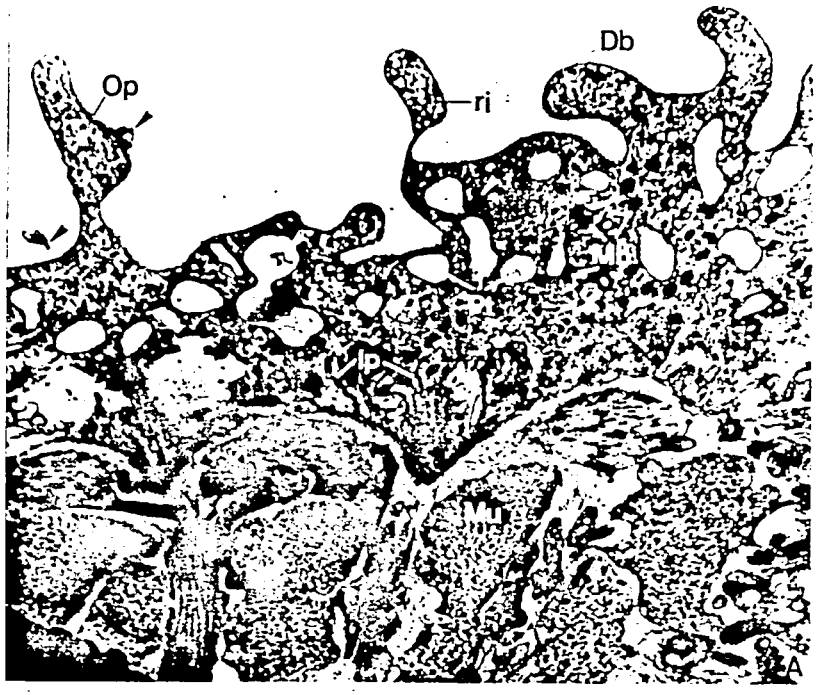
C & D) duplicate experiments on S. japonicum (Chinese), in "D" different lots of mouse antisera (1, 2, 3, 4) were used.

PLATE 3 Immunoprecipitations of ^{125}I-labelled surface proteins from adult male S. japonicum (Philippine) (PH) and S. mekongi (ME) by ISMA, ISCH, ISPH, ISME, ISHE and IHPH. All samples were preabsorbed with normal mouse serum (NMS), and the gels used were 12.5% SDS-PAGE.

A) S. japonicum (Chinese) - for comparison

B) S. japonicum (Philippine)

C) S. mekongi - for comparison



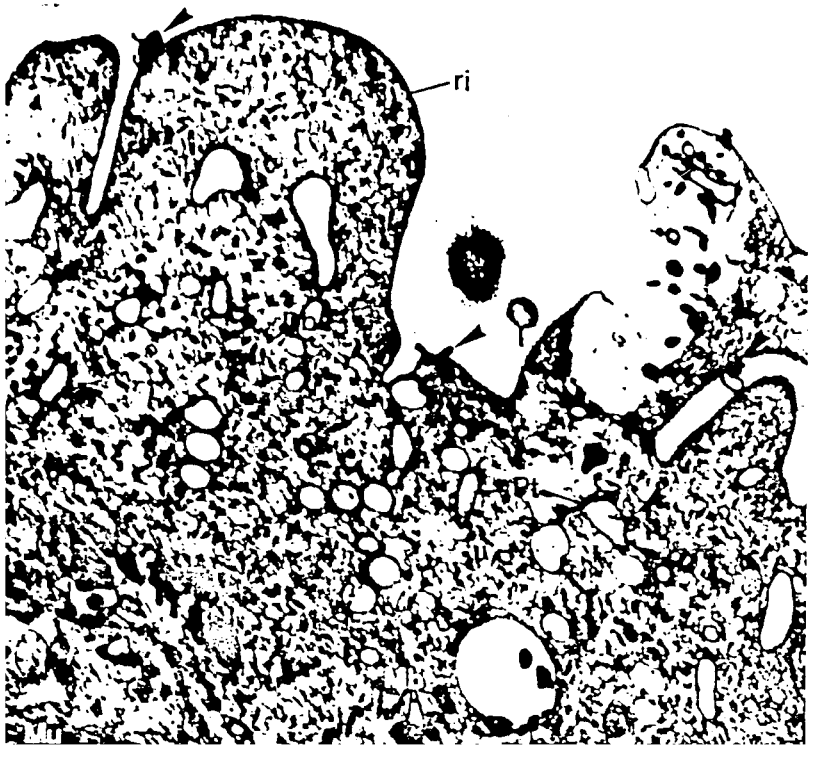


PLATE I

ELECTRON MICROSCOPIC AUTORADIOGRAPHY (EM-ARG) TO DETECT THE ^{125}I-LABELLING SITES ON THE TEGUMENT OF *S. JAPONICUM* (CHINESE & PHILIPPINE) FOLLOWING IODINATION BY IODOGEN METHOD

A) EM-ARG of the tegument of an adult male *S. japonicum* (Chinese). Notice that most silver grains (arrow heads) lie either just outside or on the membrane of pits (Pt) and ridges (Ri). X 20,000.

B) EM-ARG of the tegument of an adult male *S. japonicum* (Philippine). All silver grains (arrow heads) in the micrograph superimpose on the membrane of pits (Pt) and ridges(ri). X 35,000.

Abbreviations : Mb - membranous bodies; Db - discoid body; Ip - infolding of the basal membrane, Mu - muscle.

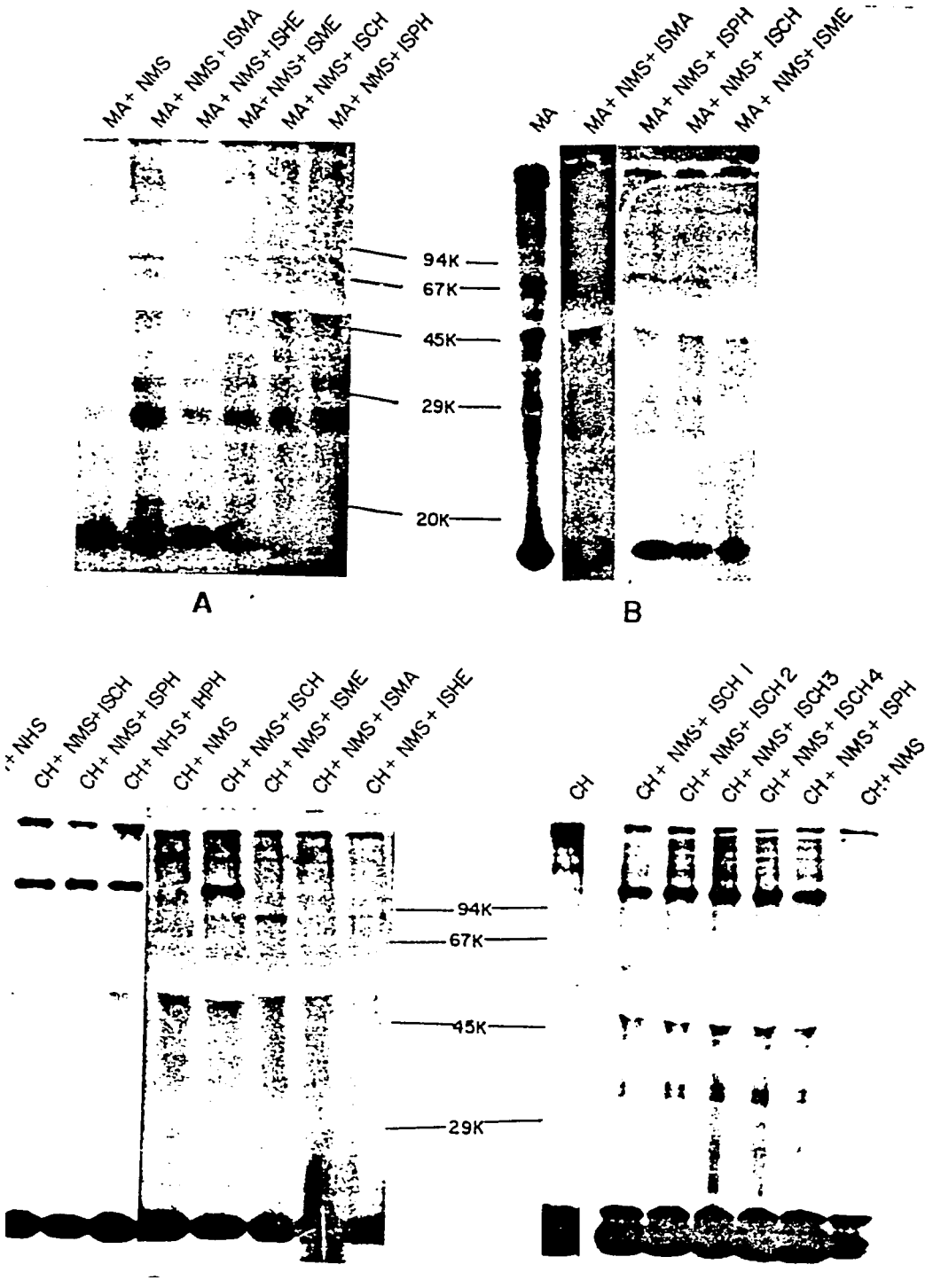


PLATE 2

IMMUNOPRECIPITATION PATTERNS OF ^{125}I-LABELLED SURFACE PROTEINS FROM ADULT MALE *S. MANSONI* (MA), *S. JAPONICUM* (CHINESE - CH) BY MOUSE IMMUNE SERA AGAINST *S. MANSONI* (ISMA), *S. JAPONICUM* (CHINESE & PHILIPPINE - ISCH & ISPH), *S. HEMATOBIUM* (ISHE), *S. MEKONGI* (ISME), AND HUMAN IMMUNE SERA AGAINST *S. JAPONICUM* (PHILIPPINE - IHPH). THE GELS USED IN ALL CASES WERE 12.5% SDS-PAGE, AND ALL SAMPLES WERE PREABSORBED WITH NON-IMMUNE MOUSE SERUM (NMS).

A & B - duplicate experiments on *S. mansoni*.

C & D - duplicate experiments on *S. japonicum* (Chinese), in "D" different lots of mouse antisera (1, 2, 3, 4) were used.

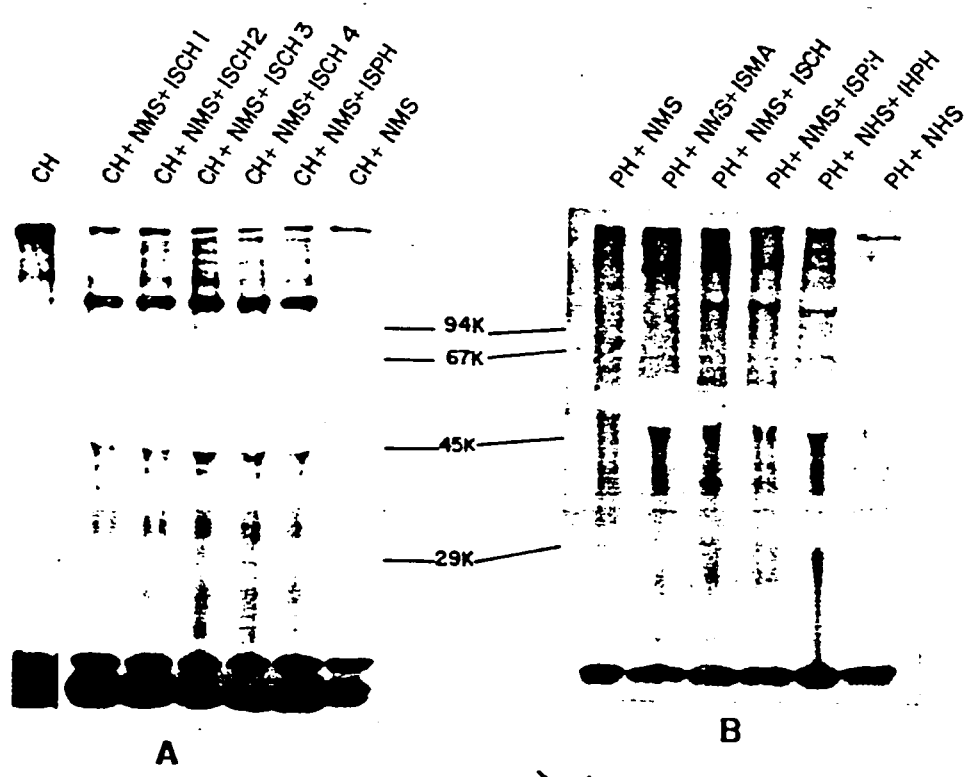


PLATE 3

IMMUNOPRECIPITATIONS OF ^{125}I-LABELLED SURFACE PROTEINS FROM ADULT MALE *S. JAPONICUM* (PHILIPPINE) (PH) AND *S. MEKONGI* (ME) BY ISMA, ISCH, ISPH, ISME, ISHE AND IHPH. ALL SAMPLES WERE PREABSORBED WITH NORMAL MOUSE SERUM (NMS), AND THE GELS USED WERE 12.5% SDS-PAGE.

A) *S. japonicum* (Chinese) - for comparison

B) *S. japonicum* (Philippine)

C) *S. mekongi*

WORKPLAN FOR THE NEXT PERIOD

In the next period we plan to :

1. perform immunoprecipitation experiments to identifiy major surface antigens on cercariae and schistosomulae using ^{125}I labelling.

2. perform immunoblotting experiments to identify major surface antigens of adult using freeze-thaw tegument and already prepared high-titer natural immune sera, and immune sera against adult purified membrane. The pattern of adult surface antigens as obtained by this technique will be compared with that obtained by immunoprecipitation.

3. We shall try another method of labelling surface glycoproteins of adult parasites by using NaB^3H_4/periodate oxidation and immunoprecipitation to detect directly the surface antigens which are glycoproteins.

4. As for monoclonal antibody production we have 2 positive clones of fused hypridoma and spleen cells as tested by immunoblotting against surface antigens. The first batch of spleen cells were obtained from immunizing animals with homogenized total body proteins from adult parasites. In the next period the spleen cells harvested from animals immunized with freeze-thaw tegument, and from naturally-infected cercariae and schistosomulae will be due and we hope to have more positive clone with high teter of MAB for further testing.