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PROGRESS REPORT NO. 5

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

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 Antigens 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	November 1988 - March 1989
BUDGET ALLOCATION FOR THIS PERIOD	521,300 Bahts

CONTENT

1. BACKGROUND

The grant was awarded on August 11, 1986. During the two and 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 and immunoblotting methods. 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 further work on the production of more clones of monoclonal antibodies against surface antigens which have been tested for activities by using ELIZA and immunoblotting assays.

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 and identify MAB that can react with specific surface antigens (and released antigens), which could be used for immunodiagnosis, and MAB that can kill schistosomula.

3. MATERIALS & METHODS & RESULTS

3.1 Production of monoclonal antibodies (MAB) against surface antigens of *S. japonicum* (Chinese)

Since the last progress report more hybridomas were produced in order to obtain more monoclonal antibodies of specific class, subisotype and specificity. Fusion was performed according to the previously reported method using surface antigens of adult Chinese strain *S. japonicum*. Of the 37 ELISA positive wells of hybridomas, 30 were found to produce sufficiently high antibody titer.

The hybridomas which was found to produce high antibody titer were expanded in culture and clonning was performed by limiting dilution method

Antibody production of the cloned cells were checked regularly by ELISA technique as shown in Table 1. The cloned cells which were found to produced high antibody titer were selected and expanded in culture. The cloned cells which remain survive in culture and produce high antibody titer were being recloned for further expansion in vivo and in vitro. After the antibody class and subisotypes were determined these monoclonal antibodies and the antibodies from previous experiments were used for immunoblotting assays and killing assay against schistosomula of *S.japonicum*

Table 1 ELISA assay for monoclonal antibody in the culture supernatants of hybrid cells immunized with *S. japonicum* (Chinese strain) antigens

CODE OF CLONED CELLS	7 Feb	31 Jan	30 Jan	29 Jan	26 Jan	16 Jan
Negative Control (O.D.)	0.261	0.139	0.051	0.059	0.049	0.069
Positive Control (O.D.)	2.013					
1) 2C11-1		0.289				
2C11-2		0.339				
2C11-3		0.391				
2C11-4	0.186	0.334				
2C11-5		0.308				
2C11-6	0.475	0.338				
2C11-7		0.260				
2C11-8		0.269				
2) 3C5-1	0.125	0.120				
3C5-2	0.238	0.115				
3C5-3	0.262	0.127				
3C5-4	0.210	0.114				
3C5-5	0.252	0.136				
3) 3C10-1	0.300					
3C10-2	0.298					
3C10-3	0.196					
3C10-4	0.259					
3C10-5	0.349					
3C10-6	0.279					
3C10-7	0.213					
3C10-8	0.168					

CODE OF CLONED CELLS	7 Feb	31 Jan	30 Jan	29 Jan	26 Jan	16 Jan
Negative Control	0.261	0.139	0.051	0.059	0.049	0.069
Positive Control	2.013					
4) 6C4-1	0.354					
6C4-2	0.228					
6C4-3	0.145					
6C4-4	0.164					
6C4-5	0.145					
6C4-6	0.365					
6C4-7	0.283					
5) 6F11-1		0.262				
6) 6G2-1		0.150				
6G2-2		0.251				
6G2-3	0.153	0.299				
6G2-4	0.155	0.314				
6G2-5	0.207	0.325				
6G2-6		0.217				
6G2-7		0.292				
6G2-8		0.245				
7) 7C5-1			0.154	0.114		0.221
7C5-2			0.127	0.115		0.329
7C5-3			0.136	0.088		0.198
7C5-4			0.128	0.123		0.195
7C5-5			0.174	0.057		0.183
7C5-6						0.213
7C5-7			0 117	0.057		0.257

CODE OF CLONED CELLS	7 Feb	31 Jan	30 Jan	29 Jan	26 Jan	16 Jan
Negative Control	0.261	0.139	0.051	0.059	0.049	0.069
Positive Control	2.013					
8) 7C6-1		0.083	0.140	0.095	0.385	
7C6-2			0.128	0.080	0.314	
7C6-3			0.115	0.071	0.253	
9) 7E9-1			0.106	0.051	0.278	
7E9-2			0.113	0.056	0.345	
7E9-3					0.158	
7E9-4			0.114		0.158	
10) 7F6-1			0.097		0.276	
7F6-2			0.112		0.348	
7F6-3			0.120		0.562	
7F6-4			0.109		0.448	
7F6-5					0.394	
7F6-6					0.627	
7F6-7		0.104	0.165	0.107	0.458	
11) 7G5-1			0.143	0.085	0.273	
7G5-2		0.114	0.199	0.092	0.225	
12) 8B4-1		0.315				
13) 8B6-5		0.293				
14) 8C9-1			0.157	0.174	0.138	0.336
8C9-2			0.139	0.121	0.117	0.419
8C9-3				0.107	0.100	0.350

CODE OF CLONED CELLS	7 Feb	31 Jan	30 Jan	29 Jan	26 Jan	16 Jan
Negative Control	0.261	0.139	0.051	0.059	0.049	0.069
Positive Control	2.013					
15) 8C11-1	0.134					
8C11-2	0.181					
8C11-3	0.263	0.309				
8C11-4	0.137	0.294				
8C11-5	0.163	0.358				
8C11-6	0.122					
8C11-7	0.118					
8C11-8	0.114					
16) 8D6-1		0.442				
8D6-3		0.303				
8D6-5		0.381				
8D6-7		0.414				
8D6-8		0.399				
17) 8E2-1		1.111				
8E2-2		1.001				
8E2-3		0.360				
8E2-4		1.192				
8E2-5		0.944				
8E2-6		0.116				
8E2-7		1.475				
8E2-8		1.376				

CODE OF CLONED CELLS	7 Feb	31 Jan	30 Jan	29 Jan	26 Jan	16 Jan
Negative Control	0.261	0.139	0.051	0.059	0.049	0.069
Positive Control	2.013					
18) 8F2-1	0.323	0.291				
8F2-2	0.326	0.331				
8F2-3	0.322	0.343				
8F2-4	0.384	0.326				
8F2-5	0.421	0.322				
8F2-6	0.208	0.248				
8F2-7	0.192	0.308				
8F2-8	0.192	0.249				
19) 8F5-1	0.168	0.275				
8F5-2	0.150	0.124				
8F5-3	0.120	0.189				
8F5-4	0.164	0.203				
8F5-5	0.210	0.134				
8F5-6	0.174	0.116				
8F5-7	0.081	0.159				
8F5-8	0.095	0.105				
8F5-9	0.135					
8F5-10	0.137					
8F5-11	0.163					
8F5-12	0.281					
20) 8F10-1	0.300					
8F10-2	0.302					
8F10-3	0.233					
8F10-4	0.111					

CODE OF CLONED CELLS	7 Feb	31 Jan	30 Jan	29 Jan	26 Jan	16 Jan
Negative Control	0.261	0.139	0.051	0.059	0.049	0.069
Positive Control	2.013					
8F10-5	0.281					
8F10-6	0.118					
8F10-7	0.226	0.232				
8F10-8	0.325	0.222				
21) 8G3-1			0.236	0.258	0.234	0.231, 2.102
8G3-2			0.206	0.335		0.124, 1.568
8G3-3			0.243	0.326		0.133, 1.901
8G3-4			0.201	0.307		0.142, 1.848
8G3-5			0.274	0.217	0.197	0.293, 1.549
22) 8G9-5		2.305	0.391		0.262	
8G9-6		1.622				
8G9-7		0.934				
23) 8G11-1				1.042		1.961
8G11-2				1.008		0.648
8G11-3				0.932		1.576
8G11-4				0.850		1.238
8G11-5			1.073	1.301		1.121
8G11-6				0.820		1.163
8G11-7				0.978		0.731
8G11-8				0.790		1.747
8G11-9			1.073	1.076		1.084
8G11-10				1.802		1.573
8G11-11				1.230		1.527

CODE OF CLONED CELLS	7 Feb	31 Jan	30 Jan	29 Jan	26 Jan	16 Jan
Negative Control	0.261	0.139	0.051	0.059	0.049	0.069
Positive Control	2.013					
24) 9B11-1	0.239					
9B11-2	0.182					
9B11-3	0.134					
9B11-4	0.119					
9B11-5	0.326					
9B11-6	0.372					
25) 9C3-1	0.283					
9C3-2	0.270					
9C3-3	0.257					
9C3-4	0.282					
9C3-5	0.270					
9C3-6	0.144					
9C3-7	0.088					
26) 9C4-1	0.219					
9C4-2	0.339					
9C4-3	0.294					
27) 9C11-1	0.130					
9C11-2	0.170					
9C11-3	0.203					
9C11-4	0.215					
28) 9E7-1	0.211					
9E7-2	0.165					
9E7-3	0.135					

CODE OF CLONED CELLS	7 Feb	31 Jan	31 Jan	29 Jan	26 Jan	16 Jan
Negative Control	0.261	0.139	0.051	0.059	0.049	0.069
Positive Control	2.013					
9E7-4	0.177					
9E7-5	0.154					
9E7-6	0.208					
9E7-7	0.192					
9E7-8	0.192					
29) 9F3-1	0.085					
9F3-2	0.076					
30) 9F8-1	0.273					
9F8-2	0.225					

3.2 Immunoblotting Assay

Clones that showed high titer of antibodies according to ELIZA result (shown in Table 1) were further tested by immunoblotting assay. In this assay the antigens from freeze-thaw adult *S. japonicum* tegument were separated by 12.5% SDS-PAGE as previously described, and blotted onto nitrocellulose paper (NC). The NC strips were incubated further in culture fluid containing monoclonal antibodies (MAB). The positive MAB that reacted with the antigens from freeze-thaw tegument were detected by incubation in rabbit anti-mouse Ig-HRP and visualized by further incubation in DAB and H_2O_2. It was found that positive band was detected in culture fluids derived from Clones 8D6; 8F2; 8F5; 8F10; 8G3-2, -3, -4, -5; 8G9, -5 while the remaining clones showed negative result. The most prominent band of antigen detected by MAB in these clones was approximately at 68,000 dalton (see Figure 1).

4. Conclusion & Remarks

So far about 40 clones of fused myeloma cells have been kept in culture. However, only 6 clones were found to contain MAB against one major band of surface antigen.

5. Workplan for the Next Period

We would like to test MAB derived from these positive clones by immunofluorescence assay to detect the location of the specific antigen on the parasite bodies that they bind to. This MAB will also be used in immune killing assay against schistosomula. In addition, in the remaining period more clones of myeloma cells will be fused and assayed for the presence of MAB against surface antigens.

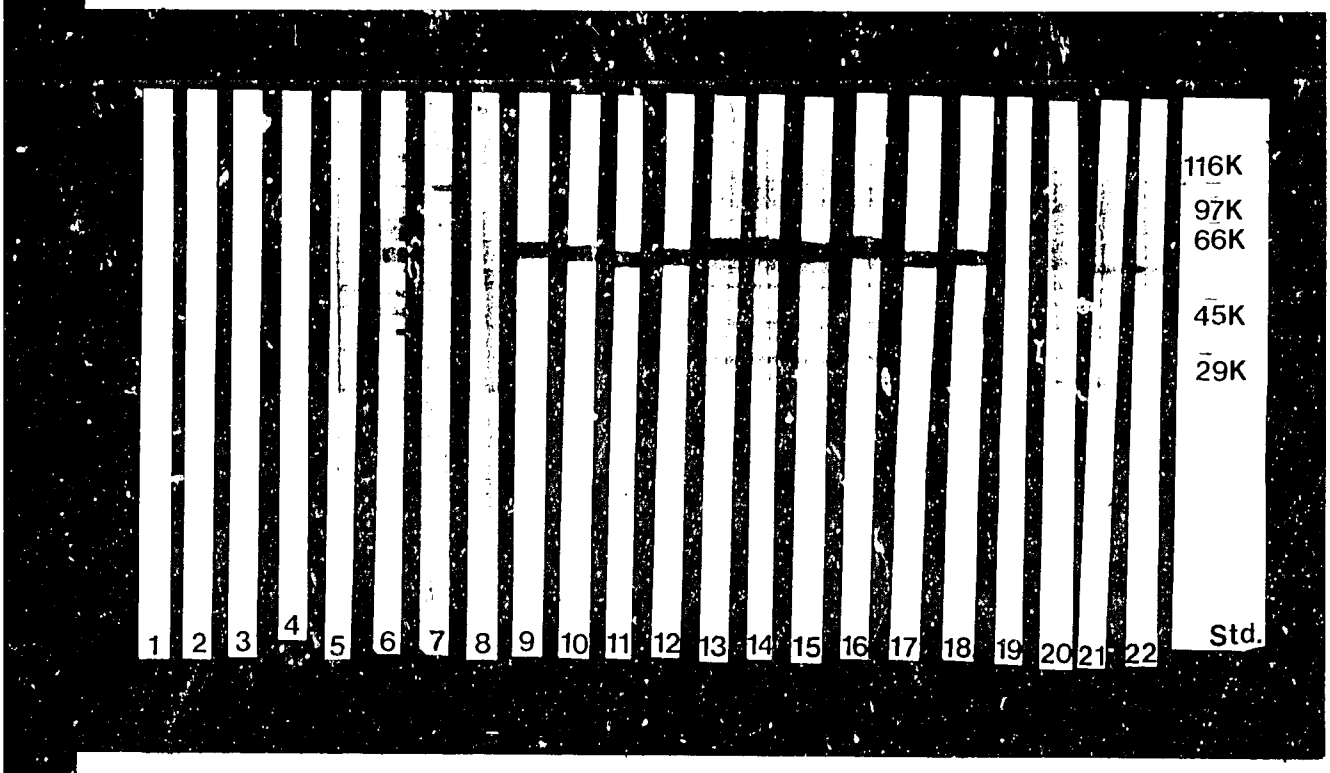

Figure 1 Immunoblotting Assays for MAB in the culture fluids of various clones of hybridoma

Lane 1	RPMI + Fetal calf serum (FES)
Lane 2	RPMI
Lane 3	FES
Lane 4	Freeze-thaw antigens (FA) + Amido black
Lane 5	FA + Normal mouse serum
Lane 6	FA + immune mouse serum against *S.japonicum* (Chinese)
Lane 7	FA + immune mouse serum against *S.mansoni*
Lane 8	FA + 6C7 - 10 fluid
Lane 9	FA + 8D6 fluid
Lane 10	FA + 8F2
Lane 11	FA + 8F5
Lane 12	FA + 8F10
Lane 13	FA + 8G3-2
Lane 14	FA + 8G3-3
Lane 15	FA + 8G3-4
Lane 16	FA + 8G3-5
Lane 17	FA + 8G9
Lane 18	FA + 8G9-5
Lane 19	FA + 8G11-1
Lane 20	FA + 8G11-2
Lane 21	FA + 8G11-5
Lane 22	FA + 8G11-10
Lane 23	Std HMW