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PROGRESS REPORT NO. 3

APPLICATION OF MONOCLONAL ANTIBODIES AGAINST
ENTAMOEBA HISTOLYTICA IN TROPICAL MEDICINE RESEARCH

A RESEARCH PROJECT

USAID/PSTC PROGRAM

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SUBMITTED BY

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PROJECT PROFILE

Country : Thailand

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PROGRESS REPORT

1. Cloning of hybrids secreting anti-E. histolytica antibodies.
2. Separation of cysts from asymptomatic amoebiasis and immunization.
3. Development of a typing scheme using IFA screening test of cysts and cultured trophozoites with panel of monoclonal antibodies against E. histolytica.
4. Isoenzyme typing of various strains of E. histolytica from patients with either invasive or asymptomatic amoebiasis.
5. Conclusion.
6. Work plan for the next period.

1. CLONING OF HYBRIDS SECRETING ANTI-E. HISTOLYTICA ANTIBODIES

We have shown in the Progress Report No.2 that oligoclonal antibodies of 13 positive hybrids were classified by the IFA test into three groups. The first group consisted of 9 hybrids, the products from which were reactive against all components of the amoebae comprising the granules (G), the membranes (M), the cytosol (C) and the released products (RP) from amoebae. The second group comprising only one hybrid had its product reacting to cytoplasmic granules only. The third group comprising 3 hybrids, the products from which reacted only to the cytosol. Eight of thirteen hybrids have been cloned by a limiting dilution technique. IFA and ELISA reactivities of the antibodies from these monoclones against two strains of E. histolytica and a Laredo strain are shown in Table 1. These clones were further expanded and cryopreserved in liquid nitrogen. Monoclonal antibodies (MAb) collected from culture supernatants were stored at -20°C or concentrated by Carbowax M20 prior to storage.

Table 1. IFA and ELISA reactivities of monoclonal antibodies against two strains of *E. histolytica* and a Laredo strain.

Designation	Group	Monoclonone No.	IFA			ELISA		
			HM-1	HK-9	Laredo	HM-1	HK-9	Laredo
F1W6	I	6C2	3+	2+	-	0.00	0.00	0.00
		6C2/8	2+	2+	-	0.00	0.00	0.00
		6C2/19	2+	2+	-	0.00	0.00	0.00
		6C14	1+	2+	-	0.00	0.00	0.00
		6C22	2+	2+	-	0.00	0.00	0.00
		6C1	3+	2+	-	0.00	0.00	0.00
		6C2/40	2+	2+	-	0.06	0.10	0.00
		6C12	1+	1+	-	0.00	0.00	0.00
		6C13	1+	1+	-	0.00	0.00	0.00
		6C18	2+	2+	-	0.00	0.00	0.00
		6C19	2+	2+	-	0.00	0.00	0.00
		6C20	1+	2+	-	0.00	0.00	0.00
		6C24	2+	2+	-	0.00	0.00	0.00
		6C26	2+	2+	-	0.00	0.00	0.00
F1W19	I	19C11	2+	2+	-	0.00	0.00	0.00
		19C13	3+	2+	-	0.00	0.00	0.00
		19C13/15	3+	2+	-	0.00	0.00	0.00
		19C13/17	3+	2+	-	0.00	0.00	0.00
		19C3/18	3+	3+	-	0.00	0.00	0.00
		19C3/20	3+	3+	-	0.00	0.00	0.00

Table 1. IFA and ELISA reactivities of monoclonal antibodies against two strains of E. histolytica and a Laredo strain.

Designation	Group	Monoclonone No.	IFA			ELISA		
			HM-1	HK-9	Laredo	HM-1	HK-9	Laredo
		19C1	1+	-	-	0.00	0.00	0.00
		19C4	3+	2+	-	0.00	0.00	0.00
		19C5	3+	2+	-	0.00	0.00	0.00
		19C6	2+	1+	-	0.00	0.00	0.00
		19C7	3+	2+	-	0.00	0.31*	0.00
		19C8	2+	3+	-	0.00	0.00	0.00
		19C9	2+	2+	-	0.00	0.00	0.00
		19C14	3+	2+	-	0.00	0.00	0.00
F1W35	I	35C1	3+	2+	-	0.75	0.18	0.00
		35C1/1	2+	3+	-	0.72	0.43	0.00
		35C1/2	2+	2+	-	0.76	0.45	0.00
		35C1/4	3+	2+	-	0.65	0.57	0.00
		35C1/5	3+	2+	-	0.69	0.49	0.00
		35C5	3+	1+	-	0.15	0.00	0.00
		35C3	3+	2+	-	0.06	0.00	0.00
		35C4	3+	2+	-	0.08	0.08	0.00
F1W16	I	16C1	2+	2+	-	0.15*	0.03	0.04
		16C2	1+	1+	-	0.01	0.02	0.01
		16C3	1+	1+	-	0.02	0.00	0.08
		16C7	2+	2+	-	0.04	0.00	0.00
		16C8	2+	2+	-	0.00	0.00	0.00

2. CONCENTRATION OF AMOEBIC CYSTS FROM THE STOOL

Cysts from asymptomatic intestinal amoebiasis were separated from other faecal materials using a discontinuous percoll gradient technique of Avron et al. (Exp. Parasitol. 55:265-9, 1983) with minor modification. Briefly a 100 µl of faecal suspension in phosphate buffered saline (PBS) was placed at the bottom of a 16x125 screw-capped tube, on top of which are successive layers of 100%, 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10% Percoll in PBS. The tubes were centrifuged at 900xg for 15 min at room temperature and the cysts from each layer of Percoll counted. The cysts present between Percoll layers of 40-50% were washed 3 times in PBS and spotted onto glass microscopic slides to be tested by an IFA technique against MAb and to be used as antigen for immunization. Bacteria associated with the cysts were eliminated by antibiotics comprising 1,000 unit penicillin G sodium and 2,000 µg streptomycin per ml of PBS. One balb/C mice was immunized with sonicated cysts, whilst the other two were immunized with nonsonicated cysts. Unfortunately, all mice died after the third immunization dose.

3. DEVELOPMENT OF A TYPING SCHEME FOR DIFFERENTIATION BETWEEN 'INVASIVE' AND 'NONINVASIVE' STRAINS OF E. HISTOLYTICA

Development of a typing scheme for E. histolytica by MAb was made against three isolates of pathogenic E. histolytica (HTH-33:MUTM, HTH-34:MUTM and HTH-35:MUTM), two isolates of non-pathogenic E. histolytica (HTH-37:MUTM and HTH-45:MUTM) and one isolate of amoeba which could not be precisely grouped as either E. histolytica or E. histolytica-like amoeba (HTH-29:MUTH). Two

strains of axenically cultivated E. histolytica belonging to zymodeme II (HM-1:IMSS and HK-9) were also tested.

Thirty MAb were tested against cultured trophozoites of the first 5 isolates and 2 other axenically cultured E. histolytica. Only 6 MAb (35C1, 35C1/4, 19C13, 6C2/40, 35C5 and 6C2/8) were tested against cysts of the HTH-45:MUTM. Our preliminary result (table 2) showed that only 4 MAb reacted to at least 5 strains of amoebae tested (6C1, 19C4, 19C11 and 19C13), 14 MAb reacted to 4 strains (6C2, 6C12, 6C13, 6C14, 6C18, 6C19, 6C20, 6C22, 6C28, 19C7, 19C9, 19C14, 35C3 and 35C4), 4 MAb reacted to 3 strains (6C24, 19C5, 19C8 and 35C1), the other 8 MAb did not react to any of 5 cultured strains at all (19C1, 16C6, 35C5, 16C1, 16C2, 16C3, 16C7 and 16C8), and 6 MAb did not react at all against cysts of the HTH-45:MUTM (35C1, 35C1/4, 19C13, 6C2/40, 35C5 and 6C2/8). The IFA reactivities were directed mostly to granules. Seven MAb had additional reaction to the membrane of E. histolytica. (35C3 vs HTH-33:MUTM; 6C12 and 35C1 vs HTH-34:MUTM; 6C20, 19C7, 35C1 and 35C4 vs HTH-35:MUTM). It was noted that MAb from clones 6C14, 35C3 and 35C4 did not react at all to non-pathogenic strains of the HTH-37-MUTM. These MAb will be retested against a larger numbers of known pathogenic and nonpathogenic E. histolytica to be certain whether they are specific only to pathogenic strains.

Table 2. *IFA reactivities of some MAb against cultured trophozoites
of *E. histolytica*

Hybridoma line code	HTH-29	HTH-33	HTH-34	HTH-35	HTH-37	HTH-45 *	HM-1	HK-9	
F1W	6C1	1+	1+	1+	2+	2+	ND	3+G	2+G
	6C2	-	1+	1+	2+	1+	-	3+MG	2+MG
	6C2/8	ND	ND	ND	ND	ND	-	2+MG	2+MG
	6C12	-	2+	1+M	1+	1+	ND	1+G	1+G
	6C13	-	2+	1+	1+	1+	ND	1+G	1+G
	6C14	1+	2+	2+	1+	-	ND	1+MG	2+MG
	6C18	-	2+	1+	1+	1+	ND	2+G	2+G
	6C19	-	2+	1+	1+	1+	ND	2+G	2+G
	6C20	1+	1+	-	1+M	1+	ND	1+G	2+G
	6C22	-	1+	1+	1+	1+	ND	2+MG	2+MG
	6C24	-	2+	-	1+	1+	ND	2+G	2+G
	6C26	-	2+	1+	1+	1+	ND	2+G	2+G
F1W	19C1	-	-	-	-	-	ND	1+G	-
	19C4	1+	1+	1+	2+	1+	ND	3+G	2+G
	19C5	-	-	1+	1+	1+	ND	3+G	2+G
	19C6	-	-	-	-	-	ND	2+G	1+G
	19C7	-	1+	1+	1+M	1+	ND	3+G	2+G
	19C8	-	1+	-	1+	1+	ND	3+G	2+G
	19C9	-	1+	1+	1+	1+	ND	2+G	2+G
	19C11	1+	2+	1+	1+	1+	ND	3+GM	2+GM
	19C13	1+	2+	1+	1+M	1+	-	3+GM	2+GM
	19C14	-	1+	1+	1+	1+	ND	3+G	2+G
F1W	35C1	-	2+	2+M	2+M	-	-	3+MG	2+MG
	35c1/4	ND	ND	ND	ND	ND	-	3+MG	ND

Table 2. IFA reactivities of some MAb against cultured trophozoites
of *E. histolytica*

Hybridoma line code	HTH-29	HTH-33	HTH-34	HTH-35	HTH-37	HTH-45 *	HM-1	HK-9
35C3	1+	2+M	1+	2+	-	ND	3+G	2+G
35C4	1+	1+	1+	1+M	-	ND	3+G	2+G
35C5	-	-	-	-	-	-	3+GM	1+G
F1W 16C1	-	-	-	-	-	ND	2+CG	2+CG
16C2	-	-	-	-	-	ND	1+CG	1+CG
16C3	-	-	-	-	-	ND	1+CG	1+CG
16C7	-	-	-	-	-	ND	2+CG	2+CG
16C8	-	-	-	-	-	ND	2+CG	2+CG

* = cyst not cultured trophozoite

ND = Not Done

- = Negative

4. ISOENZYME TYPING OF E. HISTOLYTICA

Based on 4 isoenzymes, namely HK, ME, GPI and PGM, twenty-four E. histolytica isolates from patients attending hospital clinics in Bangkok have been typed using the technique developed by Sargeant *et al.* (Trans.Roy.Soc.Trop.Med.Hyg.: 78:96-101, 1984). Enzyme lysate from HM-1:IMSS strain of pathogenic E. histolytica was used as zymodeme II markers (Fig. 1). It was found that there were 15 pathogenic E. histolytica zymodeme II, 1 nonpathogenic E. histolytica zymodeme I, 3 nonpathogenic and 3 pathogenic E. histolytica with unidentified zymodeme and 2 unidentified amoebae. These results will be consulted with Mr. Peter G. Sargeant who is the expert on zymodeme typing of E. histolytica.

5. CONCLUSION

Development of a typing scheme for differentiation between invasive and non-invasive strains of E. histolytica by 30 MAb was made against 3 isolates of pathogenic E. histolytica, two isolates of nonpathogenic E. histolytica and one amoebae of unknown species. It was noted that MAb from clones 6C14, 35C3 and 35C4 did not react at all to one nonpathogenic strain of E. histolytica. These MAb will be retested against a larger numbers of known pathogenic and nonpathogenic E. histolytica to be certain whether they are specific only to pathogenic strains.

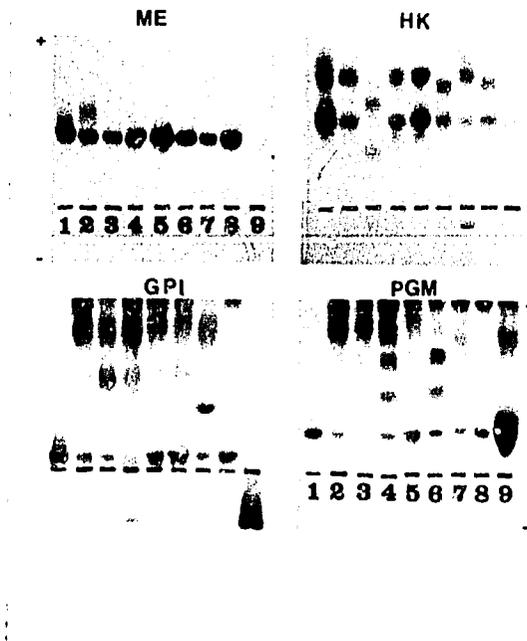


Fig. I Zymogrames of hexokinase (HK), malic enzyme (ME), glucose phosphate isomerase (GPI) and phosphoglucosmutase (PGM) of *E. histolytica* after starch gel electrophoresis

- 1 = HTH-35:MUTM
- 2 = HM-1:IMMS
- 3 = HTH-37:MUTM
- 4 = HTH-38:MUTM
- 5 = HTH-39:MUTM
- 6 = HTH-41:MUTM
- 7 = HTH-31:MUTM
- 8 = HTH-44:MUTM
- 9 = HTH-38:MUTM (faecal specimen)

6. WORK PLAN FOR THE NEXT PERIOD

- 1) More fusion and cloning.
- 2) Isotyping of monoclonal antibodies by a dot immunobinding assay.
- 3) Western blot analysis of E. histolytica antigens.
- 4) Typing scheme for differentiation between invasive and non-invasive strains of E. histolytica isolated from symptomatic and asymptomatic patients with amoebiasis.
- 5) Development of a technique for antigen detection in clinical specimens (EIA of amoebiasis using polyclonal, oligoclonal and monoclonal antibodies).