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AGENCY FOR INTERNATIONAL DEVELOPMENT
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DATE: 7/11/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 6.419
PR #4

Attachment

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PROGRESS REPORT NO. 4

APPLICATION OF MONOCLONAL ANTIBODIES AGAINST *ENTAMOEBA HISTOLYTICA* IN TROPICAL MEDICINE RESEARCH

A RESEARCH PROJECT

USAID/PSTC PROGRAM

GRANT NO. 936-5542-G-00-6029-00

SUBMITTED BY

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APPLICATION OF MONOCLONAL ANTIBODIES AGAINST *ENTAMOEBA HISTOLYTICA* IN TROPICAL MEDICINE RESEARCH

PROJECT PROFILE

Country : Thailand

Grant No. : 936-5542-G-00-6029-00

Program : Program on Science and Technology Cooperation

Project Title : Application of monoclonal antibodies against *Entamoeba histolytica* in tropical medicine research

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Total Project Budget: U.S.$ 150,000

Reporting Period : November 1987 - March 1988

OBJECTIVES

1. To type pathogenic *E. histolytica* by using mouse MABs.
2. To develop diagnostic tests for amoebic infection using mouse MABs.

PROGRESS REPORT

1. More fusion and cloning of hybrids secreting anti-*E. histolytica* antibodies.
2. Isoenzyme typing of various strains of *E. histolytica* from stool cultures and pus specimens.
3. Typing scheme for differentiation between pathogenic and non-pathogenic strains of *E. histolytica*.
4. Establishment of monoxenic cultures of *E. histolytica*.
5. Early development of a technique for antigen detection.
6. Conclusion.
7. Work plan for the next period.

1. MORE FUSION AND CLONING OF HYBRIDS SECRETING ANTI-*E. HISTOLYTICA*

One more fusion of spleen from balb/c mouse immunized with HM-1:IMSS strain of pathogenic *E. histolytica* and SP2/0 myeloma cells was performed according to the technique of Galfre and Milstein (1982). Up to now, thirty one hybrids with activities against the HM-1:IMSS strain of *E. histolytica* have been produced. Based on their immunofluorescent reactivities, these antibodies were arbitrarily classified into 4 groups. The first group consisting of nine hybrids reacted to all components of amoebic trophozoites comprising the cytoplasmic granule (G), the membrane (M), the cytosol (C) and the released products (RP). The second group comprising two hybrids reacted only to the cytoplasmic granules (G), the third group consisting of 9 hybrids reacted only to the cytosol (C) and the fourth group (11 hybrids) reacted to membrane and cytosol (C) (Table 1.). Five hybrids of group I, one hybrid of group II, five hybrids of group III and five hybrids of group IV were cloned by a limiting dilution technique. These subclones were retested again by IFA against HM-1:IMSS strain of *E. histolytica*.

2. ISOENZYME TYPING OF *E. HISTOLYTICA*

Zymodeme typing of *E. histolytica* was carried out according to the technique described by Mr. P.G. Sargeaunt, London School of Hygiene and Tropical Medicine (1984). Of 38 isolates tested, 21 belonged to pathogenic zymodemes (19 zymodeme II, 1 zymodeme XIV and 1 zymodeme XIX), 13 to non-pathogenic zymodeme I, 2 to non-*E. histolytica* amoeba and the remaining two were un-typable.

TABLE 1. INDIRECT FLUORESCENT ANTIBODY (IFA) OF OLIGOCLONAL ANTIBODIES FROM 31 HYBRIDS AGAINST HM-1:IMSS STRAIN OF *E. HISTOLYTICA*.

GROUP	STAINING PATTERN	DESIGNATION	IFA AGAINST HM-1:IMSS
I	Generalized staining of amoeba including the granules (G), cytosol (C), plasmalemma, internal membrane (M), and released produces (RP)	Eh20, Eh95	1+
		Eh6, Eh15, Eh19, Eh35	2+
		Eh16, Eh61, Eh72	3+
II	Cytoplasmic granules (G)	Eh65	1+
		Eh207	2+
III	Cytosol (C)	Eh23, Eh30, Eh52, Eh128, Eh138, Eh159	1+
		Eh131, Eh139	2+
		Eh199	3+
IV	Membrane (M) and cytosol (C)	Eh107, Eh116, Eh134, Eh135	
		Eh140, Eh152, Eh251	2+
		Eh106, Eh115, Eh191, Eh198	3+

3. TYPING SCHEME FOR DIFFERENTIATION BETWEEN PATHOGENIC AND NON-PATHOGENIC STRAINS OF *E. HISTOLYTICA* WITH MAB

IFA screening test of cultured trophozoites of *E. histolytica* with 37 MAb were tested against 3 pathogenic zymodemes of type II (HTH-33:MUTM, HTH-34:MUTM and HTH-35:MUTM), 2 non-pathogenic zymodemes of type I (HTH-37:MUTM and HTH-45:MUTM), and one non-*E. histolytica* amoeba (HTH-29:MUTM). The result is shown in Table 2. It was shown that Eh35C1 reacted to all 3 pathogenic zymodemes and not to 2 other non-pathogenic zymodemes (Fig. 1).

Fig. 1 IFA reactivity of Eh35C1 against HTH-33:MUTM.

TABLE 2. IFA REACTIVITIES OF 37 MAB AGAINST CULTURED E. HISTOLYTICA

MAB	Strains	HTH-29	HTH-33	HTH-34	HTH-35	HTH-37	HTH-45	HM-1	HK-9
	Zymodeme		II	II	II	I	I	I	I
Eh6C1		1+	1+	1+	2+	2+	1+	3+	2+
Eh6C2		-	1+	1+	2+	1+	1+	3+	2+
Eh6C2/8		ND	ND	ND	ND	ND	-	2+	2+
Eh6C12		-	2+	1+	1+	1+	-	1+	1+
Eh6C13		-	2+	1+	1+	1+	-	1+	1+
Eh6C14		1+	2+	2+	1+	-	-	1+	2+
Eh6C18		-	2+	1+	1+	1+	-	2+	2+
Eh6C19		-	2+	1+	1+	1+	1+	2+	2+
Eh6C20		1+	1+	-	1	1+	1+	1+	2+
Eh6C22		-	1+	1+	1+	1+	-	2+	2+
Eh6C24		-	2+	-	1+	1+	-	2+	2+
Eh6C26		-	2+	1+	1+	1+	-	2+	2+
Eh19C1		-	-	-	-	-	-	1+	-
Eh19C4		1+	1+	1+	2+	1+	-	3+	2+
Eh19C5		-	-	1+	1+	1+	-	3+	2+
Eh19C6		-	-	-	-	-	-	2+	1+
Eh19C7		-	1+	1+	1+	1+	-	3+	2+
Eh19C8		-	1+	-	1+	1+	-	3+	2+
Eh19C9		-	1+	1+	1+	1+	-	2+	2+
Eh19C11		1+	2+	1+	1+	1+	-	3+	2+
Eh19C13		1+	2+	1+	1+	1+	-	3+	2+
Eh19C14		-	1+	1+	1+	1+	-	3+	2+
Eh35C1		-	2+	2+	2+	-	-	3+	2+
Eh35C1/4		ND	ND	ND	ND	ND	-	3+	ND
Eh35C3		1+	2+	1+	2+	-	-	3+	2+
Eh35C4		1+	1+	1+	1+	-	-	3+	2+
Eh35C5		-	-	-	-	-	-	3+	1+
Eh16C1		-	-	-	-	-	-	2+	2+
Eh16C2		-	-	-	-	-	-	1+	1+
Eh16C3		-	-	-	-	-	-	1+	1+
Eh16C7		-	-	-	-	-	-	2+	2+
Eh16C8		-	-	-	-	-	-	2+	2+
Eh106C1		-	ND	-	ND	ND	-	3+	3+
Eh208C1		1+	ND	-	ND	ND	4+	3+	3+
Eh208C2		-	ND	1+	-	ND	1+	3+	3+
Eh208C3		-	ND	1+	-	ND	1+	3+	3+
Eh208C6		-	ND	-	-	ND	1+	3+	3+

NOTE : MAB Eh35C1 REACTS TO ALL THREE PATHOGENIC ZYMODEMES BUT NOT TO TWO NON-PATHOGENIC ZYMODEMES.

4. ESTABLISHMENT OF MONOXENIC CULTURE OF *E. HISTOLYTICA*

Liver pus from 4 Thai patients with proven amoebic liver abscess (three from the Hospital for Tropical Diseases and 1 from Haadyai Hospital, Songkla Province) were attempted to grow *in vitro*. Continuous monoxenic culture with a critidia strain has been established only with the strain from Mr. Vasana Sriburom (HTH-56:MUTM, Fig. 2). Attempts are being made to axenize this local strain of amoebae.

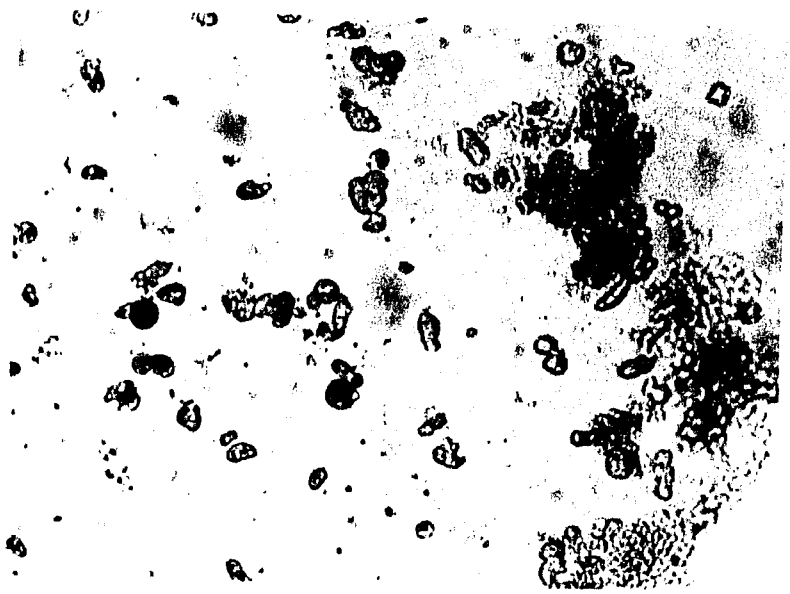

Fig. 2 Monoxenic culture of HTH-56:MUTM *in vitro*.

5. EARLY DEVELOPMENT OF A TECHNIQUE FOR ANTIGEN DETECTION

Sandwich ELISA is being developed for antigen detection. Wells of Microtiter plates were coated with IgG fraction of a rabbit raised against the HK-9 strain in carbonate-bicarbonate buffer pH 9.6. After storage at 4°C overnight, the plate was washed, and a serially-diluted suspension of the extract of the HM-1:IMSS strain of *E. histolytica* added, and the plate was further incubated. Thereafter, the plate was washed, and the $(NH_4)_2SO_4$ precipitated fraction of one MAb (Eh208C1) was added, and the plate further incubated. Thereafter, the plate was washed, and peroxidase-conjugated sheep anti-mouse IgG added, the plate was further incubated, washed and the OPD substrate was added. The colour reaction was stopped by H_2SO_4 and the result read by an ELISA reader. Up to now the test suffered from some non-specific reaction. We are trying to improve the assay technique by blocking any possible step that could give rise to the non-specific reaction.

6. CONCLUSION

6.1 Thirty one hybrids secreting antibodies against the HM-1:IMSS strain of *Entamoeba histolytica* have been produced.

6.2 Based on the staining patterns in the indirect fluorescent antibody test (IFA). These oligoclonal antibodies were classified arbitrarily into 4 groups:

Group I (8 hybrids) showed generalized staining to all components of the trophozoite comprising the cytoplasmic granule (G), the membrane (M), the cytosol (C) and the released products (RP).

Group II (2 hybrids) reacted only to the cytoplasmic granule.

Group III (9 hybrids) reacted only to the cytosol (C).

Group IV (11 hybrids) reacted to the membrane (M) and the cytosol (C).

6.3 After cloning by a limiting dilution technique, intense IFA staining (3+) were observed with MAB from 14 monoclones of 3 hybrids of group I.

6.4 Zymodeme typing of 38 isolates from Thailand showed that thirteen belong to zymodeme I, nineteen to zymodeme II, one each to zymodemes XIV and XIX, two to non-*E. histolytica* and the remaining two were un-typable.

6.5 One monoclonal antibody (Eh35C1) reacts to all three Thai isolates of pathogenic zymodemes but not to two isolates of non-pathogenic zymodeme and one isolate of non-*E. histolytica* amoeba.

6.6 Sandwich ELISA is attempting for antigen detection.

7. WORK PLAN FOR THE NEXT PERIOD

7.1 Immunization and fusion of monoxenic culture of *E. histolytica*.

7.2 Isotyping of monoclonal antibodies by a dot immunobinding assay.

7.3 Western blot analysis of *E. histolytica* antigens.

7.4 More typing of invasive and non-invasive *E. histolytica*

7.5 Detection of antigen in clinical specimens using sandwich ELISA.