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PROGRESS REPORT NO. 6

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

A RESEARCH PROJECT

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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 researc

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OBJECTIVES

1. To type pathogenic *E. histolytica* by using mouse MABs.
2. To detect amoebic antigen in clinical specimens by using mouse MABs.

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PROGRESS REPORT

1. Monoclonal antibodies against monoxenic culture of local strain of *E. histolytica*.
2. Isotyping of mouse MABs.
3. Isoenzyme typing of *E. histolytica*.
4. Sandwich ELISA technique for detection of *E. histolytica* antigen.

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1. MONOCLONAL ANTIBODIES AGAINST MONOXENIC CULTURE OF LOCAL STRAIN OF *E. HISTOLYTICA*

Immunization schedule of three BALB/c mice with monoxenic culture of HTH-56:MUTM strain of pathogenic *E. histolytica* was the same as reported in the Progress Report No. 2, except that the immunizing doses were equivalent to $1x10^5$, $2x10^5$ and $4x10^5$ for the first three doses, respectively and $9x10^5$ and $1x10^6$ for the last two doses. From three immunized mice, the same IFA titers of 1:5120, 1:5120 and 1:2560 against the homologous (HTH-56:MUTM) and the heterologous strains (HM1:IMSS) were obtained. Two mice with the titers of 1:5120 were subsequent selected for fusion. These mice were immunized intravenously with $6x10^5$ trophozoites of live *E. histolytica*. Three days after the last immunizing dose, the spleen of one BALB/c was fused with Sp2/0 myeloma cells using the technique of Galfre and Milstein (1982). Two hundred and thirty two of 540 microwells plated (42.96%) contained hybrid colonies, of which 149 (64.22%) and 97 (41.8%) yielded sypernatant fluids that were positive by IFA against the HTH-56:MUTM and the HM-1:IMSS strains respectively. Only one staining pattern against the cytoplasm, granules and membrane of fixed trophozoites were observed. The hybrids with high IFA titer were subsequently selected and cloned by a limiting dilution technique and 16 monoclones with high IFA titer were obtained. Like their predecessors, the products from these monoclones exhibited the same staining pattern.

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2. ISOTYPING OF MOUSE MABs

Classes and subclasses of these 16 MABs against local strain of pathogenic E. histolytica were determined by using mouse hybridoma isotyping kit from Calbiochem Immunochemicals. It was found that 12 clones produced IgG1 subclass, 2 clones produced IgG1 and IgM, one clone produced IgM, IgG1 and IgA and remaining clone produced IgM, IgG1 and IgG3. Obviously, only the first 12 clones producing IgG1 subclass are most likely be monoclones, and the remaining 4 are mixtures of clones.

3. 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 fifty isolates tested, 27 belonged to pathogenic zymodemes (20 zymodeme II, 1 zymodeme XIV and 1 zymodeme XIX), 19 to non-pathogenic zymodeme I, 2 to non-E. histolytica amoeba and the remaining two were untypable.

4. SANDWICH ELISA TECHNIQUE FOR DETECTION OF E. HISTOLYTICA

A modification to the a MAE-based sandwich ELISA for the detection of E. histolytica antigen described in the fifth report was made by direct application of peroxidase-conjugated rabbit anti-E. histolytica in place of a sequential application of rabbit anti-E. histolytica and peroxidase-conjugated swine anti-rabbit immunoglobulins. The IgG fraction of rabbit anti-E. histolytica strain HM1-IMSS (Ra-a-HM1) was prepared by protein-A

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Sepharose chromatography, and conjugated with horseradish peroxidase (HRP)(type VI, Sigma Chemical Co., USA) by using the two-step glutaraldehyde coupling procedure of Avrameas et al., (Scand. J. Immunol., Suppl. 7, 7, 1978). Preliminary titration of this HRP-conjugated Ra-a-HM1 IgG showed that the dilution of 1:400 was optimum (Fig. 1). An assay procedure for antigen detection was developed initially with rabbit polyclonal antibody. A polystyrene plate (Immulon, Dynatech, USA) was first coated with 100 µl of varying concentrations (100, 80, 60, 40, 20, 10 and 5 µg) of the IgG fraction of Ra-a-HM1 in a carbonate-bicarbonate buffer pH 9.6 and incubated at 37°C for 1 hour and 1°C overnight. The plate was washed with 0.15M phosphate-buffered saline pH 7.2 containing 0.05% Tween 20 (PBS-T) five times each with three min soaking. The plate was then blocked with 100 µl of PBS-T containing 0.1% gelatin and 1% bovine serum albumin (PBS-T-G-B) at room temperature for 30 min. Thereafter, 100 µl of a serial two dilutions of the saline extract of strain HM1:IMSS with the initial concentration of 100 µg/ml in PBS-T was added followed by incubation at room temperature for one hour. The plate was then washed with PBS-T and blocked with PBS-T-G-B as mentioned above. After further washing, 100 µl of HRP-conjugated Ra-a-HM1 in PBS-T at 1:400 dilution was added followed by incubation at room temperature for one hour. The plate was washed and 100 µl of O-phenylene diamine solution added. After incubation at room temperature for 30 min at room temperature, the reaction was stopped by adding 50 ul of 1N H_2SO_4 and the result read with a Multiskan ELISA reader (Titertrek) at 492 nm. The result (Fig. 2) showed that 10 µg of

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IgG fraction of Ra-a-BM1 was optimum for plate coating and this assay could detect the homologous antigen as small as 3.2 μg per ml. An attempt was being made to use anti-E. histolytica MAB to replace the IgG fraction of Ra-a-BM1. With the 50% NH_4SO_4 precipitated fraction of MAB 203 which previously gave satisfactory result, no color reaction was apparent. We are now testing the IgG fractions from two other MABs (35C1 and 65B8) to see whether they could be better utilized in the assay.

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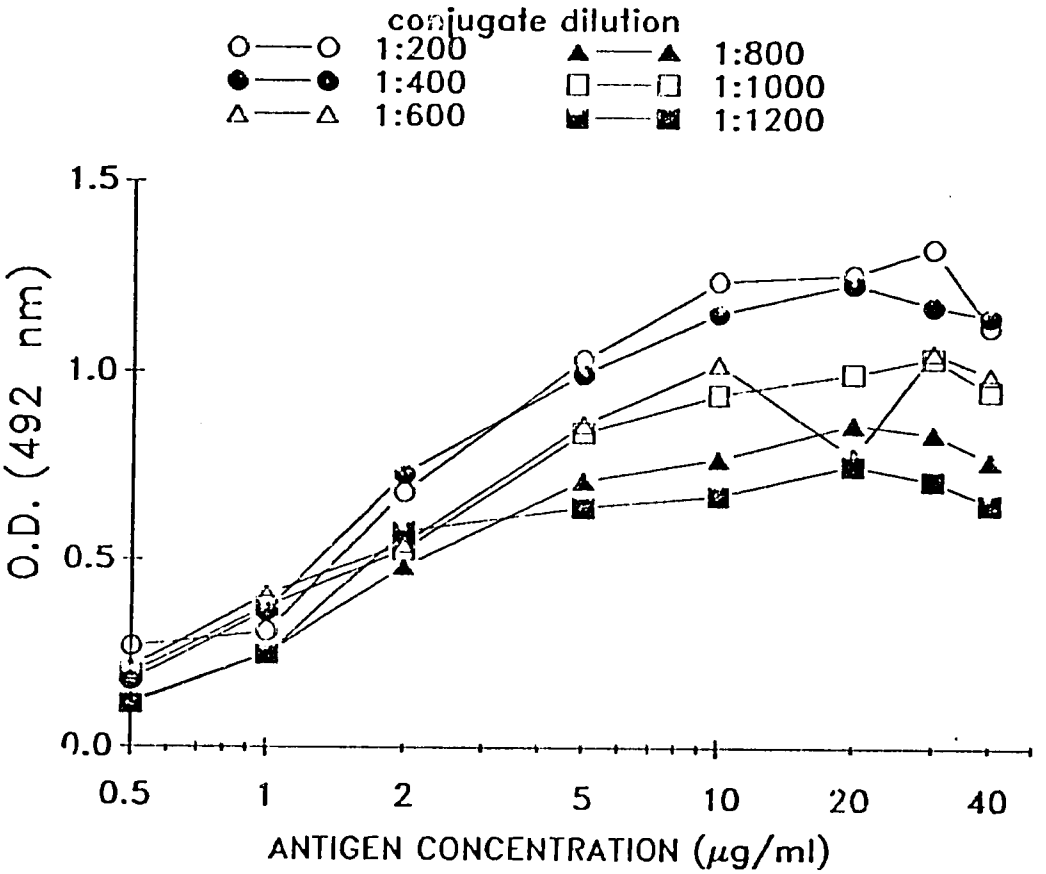

Fig. 1. Titration of peroxidase-conjugated rabbit anti-Entamoeba histolytica (HRP-Ra-a-HM1). The plate was coated with varying concentrations of the 0.15 M NaCl extract of the HM1:IMSS strain of E. histolytica, washed and added varying dilutions of HRP-Ra-a-HM1 in PBS-T. After incubation at room temperature for 1 hour, the plate was washed and O-phenylene diamine substrate, the reaction stopped with H_2SO_4 and the result read by an ELISA reader.

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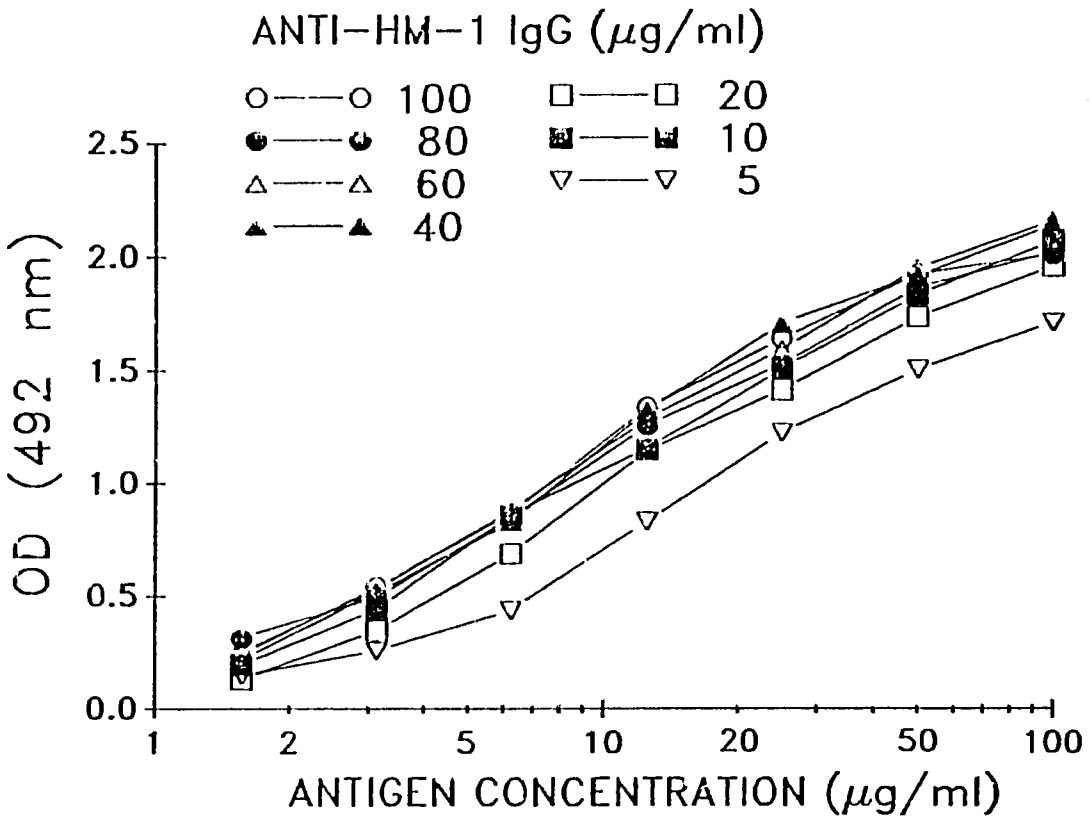

Fig. 2. Titration for optimum concentration of Ra-a-HM1 to coat plate in the detection of Entamoeba histolytica antigen. The concentration of 10 ug/ml of Ra-a-HM1 was optimum and the sensitivity of the assay was 3.1 μg/ml.

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