A STUDY OF FLUORESCENT ANTIBODIES AGAINST ENDAMOEBA HISTOLYTICA

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INTRODUCTION

Amoebiasis is not uncommon in Singapore. The bacteriology section in the department of Pathology has been isolating E. histolytica by culture from faeces and pus from liver abscesses with very good results for more than ten years. The culture medium used was introduced by Dr. L.S. da Silva in 1956 and later modified by Teo in 1963. Even with a good culture technique and very successful yield, it has not been possible to produce a pure E. histolytica antigen for use in serological tests. The E. histolytica will not multiply unless in the presence of bacteria. In a mixture of amoebae, bacteria, charcoal and starch granules, there is no way of separating out the amoeba for use as a pure antigen.

Using the indirect fluorescent antibody test described by A.L. Jeanes (1966) it has been possible to detect antibodies against E. histolytica in the patients' sera with the impure amoebic antigen that we have.

MATERIALS AND METHODS

Preparation of antigen slides

1. A strain of E. histolytica isolated from a patient was maintained in our biphasic amoebic culture medium (Yu and Teo, 1970) and subcultured every other day.
2. The amoebae were withdrawn from the bottom of four 48-hour culture tubes. This suspension was centrifuged at 2,000 rpm.(1000g.) for 10 mins. The supernatant was discarded and the sediment thoroughly resuspended in 10 mls. of phosphate buffered saline pH7.2. This was allowed to stand for two minutes, for the big debri particles to settle. The supernatant was then carefully withdrawn with a pasteur pipette. This suspension of amoebae was then centrifuged at 2,000 rpm. for 10 mins.
3. The supernatant discarded, 5 ml. of 10% formalin in saline was added to the sediment and the stirred sediment was allowed to fix for 30 mins.
4. Buffered saline was added to 10 ml. and suspension centrifuged at 2,000 rpm. for 10 mins.
5. The supernatant discarded, the sediment was resuspended in a ml. of 1% NH_4OH solution.
6. After 5 mins. the sediment was washed with buffered saline.
7. 1 ml. of 3% aqueous solution to Tween 80 was added to the sediment. After 5 mins. this was washed twice with buffered saline.
8. The final sediment was suspended in 3 ml. of buffered saline. This suspension should have about 10,000 amoebae per ml. Drops of this suspension were used for preparing the antigen slides.
9. The slides used were 3″×1″ microscope slides. Five circles of diameter 0.2″ were made on each slide in such a way so that a cover-slip could cover two circles (Fig. 1).

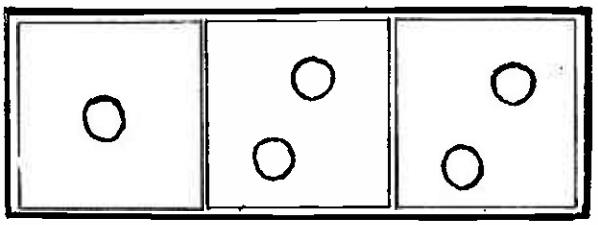

Fig. 1

The slides were placed on a hot-palte at 56°C and a drop of the amoebic suspension was made to cover the circle and rapidly dried.

10. The slides were stored at (–70°C) in a REVCO until required Slides which were kept frozen for six months still gave good results.

FLOURESCENT ANTISERUM

The fluorescein-labelled rabbit antihuman globulin used was purchased from the Wellcome Laboratories (Fluorescent antispecies globulin Antihuman MR 30).

PATIENTS' SERA

Specimen sera were diluted with phosphate buffered saline using a Takaski Microtitrator and dilutions of 1:2, 1:8, 1:16, 1:32 and 1:64 were used in each test. In a test where the amoebae were seen to fluoresce in titres of 1:16 and above was considered as a positive test.

OPTICAL SYSTEM

The optical system used was a Tiyoda 200A fluorescence microscope with a mercury vapour lamp (HBO 200) and a wide-angled Tiyoda dark-ground condenser.

SPECIMENS

The samples of serum tested were obtained from 40 healthy old ladies who were residents in a government home for the aged. They were all more than 60 years old and had no signs or symptoms of amoebiasis. All had negative stool cultures for E. histolytica.

Samples of serum obtained from 67 inmates of a mental hospital were also included. All of them had a positive stool culture of E. histolytica but none had any signs of hepatic amoebiasis. The first sample was taken one month after the positive culture and treatment for amoebiasis. Subsequently a sample of serum was taken at monthly intervals for four months.

The test was subsequently applied to 1966 routine specimens received over a period of 2½ years.

RESULTS

Table I shows the titres of 40 serum specimens from the 40 old ladies. Taking 1:16 as the positive titre, only two specimens or 5% gave a positive test titre.

TABLE I

	Negatives	Fluorescence present			Total
		1:2	1:8	1:16	
Number of specimens	33	1	4	2	40

In Table II are the results of immuno-fluorescent test performed on the specimens from 67 inmates of a mental hospital. The specimens were taken at monthly intervals starting one month after treatment for amoebiasis. In the first specimens, 20% gave a positive titre of 1:16 and above. The number of positives dropped to 17% after two months, to 12% after three months and to less than 2% after four months.

Analysing the results from our routine requests we found that out of 69 specimens where there were no signs of liver amoebiasis, we found as shown in Table III, 15 specimens where the fluorescent antibody test was positive, the E. histolytica was isolated from the faecal specimens. In 13 specimens where the test was positive the faecal culture was negative and in 41 specimens where the test was negative the faecal culture was positive.

TABLE II

Titres	Months after treatment for amoebiasis				
	One	Two	Three	Four	Five
1:256	4	5	5	1	1
1:128	3	—	—	—	—
1:64	—	—	—	—	—
1:32	5	6	—	—	—
1:16	1	—	2	—	—
1:8	—	—	1	7	—
1:4	12	—	—	—	—
1:2	22	—	—	—	—
Negative	20	54	50	50	50
Total	67	65	58	58	51
% Positive	20%	17%	12%	1.8%	1.9%

TABLE III

Number of Serum Specimens	69	100%
Faecal culture positive and F.A. positive	15	21%
Faecal culture positive and F.A. negative	41	60%
Faecal culture negative and F.A. positive	13	19%

Table IV shows 24 patients with proven hepatic amoebiasis. They either had an amoebic abscess or the amoebae were seen in liver biopsies. All except two had a positive fluorescent antibody titre. In 12 patients the faecal culture was not done, but the other 12 patients a total of 28 faecal cultures were done and no amoeba was isolated. The only two that gave a negative fluorescent antibody test, had no liver pus and the faecal culture was not done. The only indication of hepatic amoebiasis was the histology report on the needle liver biopsy.

TABLE IV

Names	Patients with hepatic amoebiasis			Faecal Culture
	Fluorescent Antibody	Liver Pus Culture	Liver Biopsy	
1. S.R.	positive×2	negative	positive	—
2. G.P.	positive	negative	—	negative×2
3. V.R.	positive×2	negative×2	positive	negative×5
4. A.S.	positive×2	negative×6	—	negative×5
5. I.B.A.	positive	negative	—	negative
6. K.Y.K.	positive	POSITIVE	positive	negative×2
7. S.K.	positive×3	negative×4	—	negative×4
8. A.T.	positive	negative×2	—	negative×3
9. M.H.	positive×2	negative×2	—	negative×2
10. Y.L.K.	positive	negative	—	—
11. C.T.	positive	negative	—	—
12. Y.T.G.	positive	POSITIVE	—	—
13. P.P.	positive	negative	—	negative
14. Y.S.N.	positive	POSITIVE	—	—
15. H.T.	positive	negative	—	—
16. C.M.	positive	negative	—	—
17. S.H.K.	positive	negative	—	—
18. K.P.S.	positive×2	negative	—	—
19. K.Y.	positive	—	positive	negative
20. S.K.	positive	—	—	negative
21. K.P.	positive	—	—	negative
22. T.K.H.	positive	—	positive	—
23. S.W.T.	negative	—	positive	—
24. N.S.K.	negative	—	positive	—

COMMENTS

The results show that the fluorescent antibody test can be most useful in confirming a case of hepatic amoebiasis. The test is positive in 20% of patients with amoebiasis without liver involvement. The fluorescent antibody titre takes about four months to disappear if a patient is treated and there is no reinfection.

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