

Comparison of Indirect Fluorescent-Antibody Amoebic Serology with Counterimmunoelectrophoresis and Indirect Hemagglutination Amoebic Serologies

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Patients ranged from those with no prior diagnosis of or suspected exposure to *Entamoeba histolytica* to those with proven amoebic liver abscesses (extraintestinal disease). A comparison of serologies from patients with proven and suspected amoebiasis or possible past exposure revealed good correlation between the indirect fluorescent antibody (IFA) procedure and the other methods used, counterimmunoelectrophoresis and indirect hemagglutination. Titers from patients with proven extraintestinal amoebiasis were in the expected high range previously reported by other authors. Patients with clinical histories suggestive of exposure to *E. histolytica* but no proven disease had lower titers which indicated possible background exposure. The IFA procedure provides a rapid method of antibody detection; results obtained on an emergency basis provide essential information in making the diagnosis of amoebic abscess, pyogenic abscess, or tumor. The IFA procedure is rapid, reliable, reproducible, and relatively inexpensive to perform, provided a good source of antigen is consistently available.

With the development of bacteria-free amoebic culture techniques (4), there have been a number of serological tests developed for the detection of *Entamoeba histolytica* infection (7). Of the serological tests available, indirect hemagglutination (IHA), latex agglutination, and counterimmunoelectrophoresis (CIE) apparently detect the same antibody (1). However, this antibody is different from that detected with the gel diffusion precipitin test and indirect fluorescent antibody test (IFA) (6). It is recommended that several serological tests be performed in suspected amoebic disease.

The CIE procedure as described in the literature has been used in this laboratory for 4 years (1, 3). After routine amoebic antigen preparation was developed in house, it was decided to make the IFA procedure available for routine testing. IFA results on patients with proven extraintestinal disease compared favorably with results for both CIE and IHA in terms of significant titers. IFA can be very valuable to clinicians by providing rapid differentiation of amoebic liver abscess (or other extraintestinal amoebic lesions) from nonamoebic etiologies. The IFA method is faster and easier to perform than the IHA procedure and can be readily adapted to "STAT" laboratory use.

MATERIALS AND METHODS

Amoebic antigen preparation. *E. histolytica* (ATCC 30015) was cultured in Diamond TPS-1 broth and

vitamin solution supplemented with 10% bovine serum (4). Organisms were harvested after 5 days by chilling the tubes in ice water for 5 min and centrifuging at $800 \times g$ for 10 min. The supernatant fluid was decanted, and the organisms were suspended in 0.85% NaCl. This washing step was repeated three times, after which the organisms were suspended in distilled water and stored at -70°C until used.

IFA procedure. Teflon-coated, 12-well (25- by 75-mm) slides (Cel-line Associates, Inc., Minotola, N.J.) were rinsed with absolute ethanol (1 min) and acetone (1 min) and then air dried. Stock antigen was diluted so that the final organism concentration was 8 to 12 organisms per $250 \times$ field. Organisms were placed in each well by using a 0.001-ml platinum bacteriological loop. Slides were dried at 37°C for 30 min and then fixed in absolute ethanol for 10 min. Prepared slides were stored at -20°C and showed no decrease in fluorescence for at least 18 months, results which are comparable to those previously reported by others (2, 3; P. Ambroise-Thomas, Ph.D. thesis, Université Claude-Bernard, Lyon, 1969).

Before use, stored slides were allowed to come to room temperature and dry for 10 min. Patient sera were initially diluted 1:50 and 1:100 with phosphate-buffered saline (0.15 M, pH 7.2). Serum portions of 10 μl were placed in each well, and these slides were incubated at room temperature in a moist chamber for 30 min. Slides were rinsed in phosphate-buffered saline for 15 min and then in a light stream of distilled water, and they were then blotted dry with bibulous paper. Fluorescein isothiocyanate anti-human globulin with Evans blue counterstain (Electro-Nucleonics Laboratories, Inc., Bethesda, Md.) (10 μl) was dispensed into each well. Slides were incubated at room

TABLE 1. Comparison of serological results of patients with extraintestinal amoebiasis

| Patient | Diagnosis | Serological result | | |
|---------|-----------------------|--------------------|---------|---------|
| | | CIE | IFA | IHA |
| 1 | Amoebic liver abscess | Positive | 1:1,600 | 1:512 |
| 2 | Amoebic liver abscess | Positive | 1:800 | 1:128 |
| 3 | Amoebic liver abscess | Positive | 1:800 | 1:512 |
| 4 | Amoebic liver abscess | Positive | 1:800 | 1:4,096 |
| 5 | Amoebic liver abscess | Positive | 1:800 | 1:512 |
| 6 | Amoebic liver abscess | Positive | 1:800 | 1:512 |
| 7 | Amoebic liver abscess | Positive | 1:800 | 1:2,000 |
| 8 | Amoebic liver abscess | Positive | 1:200 | 1:4,096 |
| 9 | Amoebic liver abscess | Positive | 1:200 | 1:4,096 |
| 10 | Amoebic liver abscess | Positive | 1:200 | 1:2,000 |
| 11 | Amoebic liver abscess | Positive | 1:100 | 1:4,096 |
| 12 | Amoeboma | Positive | 1:400 | 1:128 |

temperature in a moist chamber for 30 min, rinsed, and dried as before. Cover slips with buffered glycerol, pH 8.0, were placed over the slides, and the slides were read on a Leitz Dialux microscope equipped with Osram HBO 220 mercury energy light source, KP490 exciter filter, and K530 barrier filter. Patient sera with positive fluorescence at 1:100 were titrated out.

Controls were used with each set of patient sera: negative, weak positive (1+ at 1:400), and strong positive (3+ at 1:100). Fluorescence was graded on a scale of 1+ to 4+; 1+ indicated a thin peripheral apple-green halo around the entire organism, and 4+ indicated that the total organism was apple-green (no visible red counterstain). The endpoint was read as the highest dilution showing a 1+ reaction.

CIE procedure. CIE was performed with a Hyland power pack (electrophoresis power supply, list no. 077-922; Hyland Laboratories, Costa Mesa, Calif.) set at 40 mA for 60 min. By using a template, 3-mm wells which were 3 mm apart were punched into 1% agarose plates containing barbital buffer (0.033 M, pH 8.2).

Organisms which were stored at -70°C were freeze-thawed four times and centrifuged at $500 \times g$ for 10 min to remove particulate matter, and the supernatant was saved as a stock antigen. Stock antigen was diluted with sodium barbital buffer (0.33 M, pH 8.2) until a sharp, distinct band was produced electrophoretically by using a positive serum control.

Approximately 25- μl portions of undiluted patient sera and antigen were added in opposite wells. Positive and negative controls as well as patient sera alone (not run against antigen) were included in each run. Plates were examined for precipitin bands with the aid of a Hyland viewer. If precipitin bands with patient sera were not visible or not clear, the plate was flooded with 0.85% NaCl, stored at 4°C overnight, and reexamined the following day. A positive reaction was recorded if single or multiple precipitin bands were present between the antigen and corresponding serum wells (1, 3). Curved or hazy areas were disregarded.

IHA. The IHA serological tests were performed by the Parasitic Serology Division, Centers for Disease Control, Atlanta, Ga.

Stool specimens. Stool specimens for ova and parasite examination were submitted in polyvinyl alcohol preservative. The number of specimens submitted varied from zero to six. Formalin-ether concentration

and Trichrome permanent-stained smears were examined on every specimen submitted.

RESULTS

Patients with a clinical discharge diagnosis of extraintestinal amoebiasis (amoebic ulcer/amoeboma) had significant positive amoebic serologies (Table 1). All of the CIE serologies were positive, and the IFA results were positive with titers ranging from 1:200 to 1:1,600. Follow-up (posttherapy 8 months and 1 year, respectively) IFA titers on patients previously diagnosed and treated for amoebiasis were low (1:100 to 1:200; Table 1, patients 10 and 11). IHA titers were significant, whether the patient was previously treated or not treated, ranging from 1:128 to 1:4,096. Specimens for stool examination were submitted on 11 of 12 patients in Table 1, of which only 2 (patients 6 and 9) were found to harbor *E. histolytica*.

Patients with a diagnosis (parasitic and non-parasitic) unrelated to amoebiasis had negative or low titers by all three methods used in this study (Table 2). Stool specimens examined on patients with gastrointestinal complaints revealed no *E. histolytica*; protozoan organisms which were recovered included *Giardia lamblia*, *Entamoeba hartmanni*, and *Endolimax nana*.

DISCUSSION

In patients with amoebic abscesses, the IFA serology correlated well with both the CIE and IHA serologies in terms of significant titers. On a STAT basis, CIE and IFA can be performed much faster than IHA, and IFA results can differentiate between present and past (treated) diseases (2). The CIE and IFA serological tests for extraintestinal amoebiasis provide valuable STAT information for the clinician in differentiating the following: amoebic abscess, pyogenic abscess, and tumor. This rapid, serological dif-

TABLE 2. Comparison of serological results of patients with nonamoebiasis-related diagnosis

| Patient | Discharge diagnosis | Serological result | | |
|---------|--|--------------------|-------|-------|
| | | CIE | IFA | IHA |
| 1 | Ulcerative colitis with iron deficiency anemia | Negative | <1:50 | <1:8 |
| 2 | Appendectomy | Negative | <1:50 | <1:8 |
| 3 | Typhoid fever | Negative | <1:50 | <1:8 |
| 4 | Inflammatory bowel disease | Negative | <1:50 | <1:8 |
| 5 | Appendix perforation/abscess | Negative | <1:50 | <1:8 |
| 6 | Viral gastroenteritis | Negative | 1:50 | <1:8 |
| 7 | Carcinoma, middle ear | Negative | 1:50 | <1:32 |
| 8 | Hydatid disease/liver abscess | Negative | 1:50 | <1:32 |
| 9 | Paraovarian cysts | Negative | 1:50 | 1:32 |
| 10 | Carcinoma, liver | Negative | 1:50 | <1:8 |
| 11 | Giardiasis | Negative | 1:50 | 1:64 |
| 12 | Acute proctitis | Negative | 1:50 | <1:32 |
| 13 | Gastroenteritis | Negative | 1:50 | <1:32 |
| 14 | Systemic lupus erythematosus/tuberculosis | Negative | 1:100 | <1:32 |
| 15 | Crohn's disease | Negative | 1:100 | <1:32 |
| 16 | Polycystic ovary disease | Negative | 1:100 | <1:32 |
| 17 | Alcoholic cirrhosis | Negative | 1:100 | <1:32 |
| 18 | Cholelithiasis | Negative | 1:50 | 1:64 |
| 19 | Appendix perforation/ <i>Ascaris</i> | Negative | 1:100 | 1:32 |
| 20 | Ulcerative colitis | Negative | 1:100 | 1:32 |

ferentiation may be the basis for a surgical or nonsurgical approach to treatment.

With the exception of patients 6 and 9 (Table 1), *E. histolytica* could not be found in the stools of the majority of patients with a diagnosis of extraintestinal amoebiasis, findings similar to those confirmed by many workers (6). Patients 10 and 11 in Table 1 (amoebic liver abscess) had been previously treated. If one compares results from patient 11 (Table 1) and patients 14, 15, 16, 17, and 19 (Table 2), the importance of using two or more serological tests for amoebiasis is evident. These patients all had IFA titers of 1:100; however, only patient 11 (treated for amoebiasis) had positive CIE and IHA results.

In-house antigen preparation is time consuming but provides an excellent stable product for use in serological testing for amoebiasis. Of the number of laboratory tests available for the serological diagnosis of amoebiasis, our laboratory has chosen to use CIE and IFA methods because of the low cost, technical ease of test performance, reproducibility, and accuracy. Although these serological tests have greatly im-

proved our ability to diagnose extraintestinal amoebiasis, serological results should not be relied on exclusively to establish this diagnosis.

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