Technical method

Assessment of amoebiasis: serodiagnosis by immunofluorescence with lyophilized entamoebae

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Indirect immunofluorescence is reportedly a rapid and reliable diagnostic procedure for detecting and titrating serum antibodies to Entamoeba histolytica (Goldman, 1966; Jeanes, 1966; Boonpucknavig and Nairn, 1967). The occurrence of antibodies is of value in the diagnosis of acute infections, especially amoebic hepatitis, but not of symptomless carriers. It appears to be less reliable for colitis (Ambroise-Thomas and Truong, 1972). Growth and preparation of the Entamoeba required for antigen is difficult and retarded adoption of serological testing until the recent introduction of freeze-dried organisms ready for use as test antigen. The work to be described has confirmed and extended earlier results using immunofluorescence with freshly cultured antigen (Boonpucknavig and Nairn, 1967). A latex particle agglutination test (developed by Morris, Powell, and Elsdon-Dew, 1970), now commercially available, was assessed concurrently and found less satisfactory than the immunofluorescence test.

Materials and Methods

SERA
Human sera from clinically diagnosed cases of amoebic colitis or amoebic hepatitis were amongst those used by Boonpucknavig and Nairn (1967), and had subsequently been stored at -30°C. Control sera were from patients with other diseases.

IMMUNOFLUORESCENCE PROCEDURE
Freeze-dried amoebae (Wellcome Reagents Limited, Beckenham, Kent, England) were reconstituted with 0.5 ml distilled water and stored at 4°C. When required, smears were made on chemically cleaned glass slides using the minimum volume of suspension sufficient to cover a 2-3 mm circle. Generally six to eight smears were made on each slide thus permitting a complete serum titration to be performed on a single slide. Smears were air-dried, gently heat-fixed, then the sandwich immunofluorescence method as described elsewhere was applied (Nairn, 1969), using 0.1% Evans blue as counterstain to reduce background fluorescent staining and to give a distinct titration endpoint. FITC-labelled goat antihuman globulin was used as conjugate at a concentration of 5 mg/ml. Its FP molar ratio was 4.9 and it had been absorbed with homogenates of rat liver and intestinal tract and thoroughly washed human group AB red cell envelopes so that staining of microscopical preparations by conjugate alone was zero. Preliminary experiments had shown that treatment of amoebic smears with octan-2-ol as employed in earlier work conferred no advantage over heat fixation.

LATEX PARTICLE AGGLUTINATION
Seramoeba latex agglutination kits were obtained from Miles Laboratories, Indiana, USA, and used as directed. Sera were heat-inactivated and centrifuged before testing to remove any particulate matter. The latex suspension was thoroughly mixed with the freeze-dried amoebic antigen and left to stand for five min before use. A drop each of undiluted serum and sensitized latex suspension were placed side by side on a glass slide, mixed with a wooden stick, rotated manually for five min, then examined for agglutination.

Results
Amoebae stained as already described using positive sera showed intense green fluorescence, titres up to 640 (reciprocal of serum dilution) being recorded. Amoebae treated with negative sera at a 1 in 20 dilution showed only red fluorescence due to the Evans blue counterstain. Titres of 40 or greater were considered diagnostically significant, a titre of 20 doubtful, whilst less than 20 was recorded as negative. Two separate assessments using different batches of freeze-dried amoebae were performed and these results are summarized in the table. Titres obtained by Boonpucknavig and Nairn (1967) and the results of the latex agglutination test are included for comparison.

The stability of reconstituted organisms stored,
sterile, at 4°C for one year, was determined by comparison with freshly reconstituted amoebae using several positive and negative sera. Identical titres were obtained in all cases.

Comment

The results shown in the table illustrate the reproducibility of the immunofluorescence antibody titration in two ways. First, the four positive sera tested in both current assessments gave the same immunofluorescence titre in three cases whilst the fourth varied by only 1 doubling dilution. Secondly, comparison of the titres obtained in the current assessments with those of Boonpucknavig and Nairn (1967) shows that although titres were higher in the former they were so generally by a constant factor of 2-5. This increased sensitivity may mean that the Wellcome freeze-dried amoebae were more antigenic than freshly grown amoebae previously used, although a different criterion for determining the endpoint should not be ruled out. All control sera gave titres less than 20 with the exception of one from an asymptomatic cyst passer which had a titre of 20. It is of interest to note that although the manufacturer recommends that reconstituted amoebae be stored, frozen, for no more than one month, results reported here indicate that the antigen is sufficiently stable to permit use after one year’s storage at 4°C.

Results with latex particle agglutination conversely showed insufficient sensitivity in that five false negatives were recorded. There was also one false positive. Thus although latex agglutination is a simpler procedure for serological diagnosis, it is less reliable and lacks sufficient sensitivity for routine use. A possible explanation for this lack of sensitivity could be that agglutination techniques are especially sensitive to IgM which has not been regularly detected in amoebiasis serology, IgG being the major immunoglobulin found (Boonpucknavig and Nairn, 1967).

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References