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Immunocytochemical detection of *Entamoeba histolytica*

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Abstract

Human anti-*Entamoeba histolytica* immunoglobulin was used to detect *Entamoeba histolytica* in 74 positive samples from several different sources, using an indirect immunoperoxidase method. In 73 samples, the protozoan was easily identified. Trophozoites and cysts of all cultured *Entamoeba* strains examined were strongly stained, and as few as 3 trophozoites per microscope slide could be detected. In addition, 51 negative control samples were also tested and non-specific reactions were not observed. These preliminary results show that this method is both sensitive and specific, and can easily detect trophozoites and cysts of different *E. histolytica* strains.

Introduction

Amoebiasis, the infection caused by *Entamoeba histolytica*, is an endemic disease in several countries (Elsdon-Dew, 1968). The infection varies in severity, from asymptomatic to highly invasive, with consequences such as amoebic colitis, dysentery and liver abscess.

The detection of *E. histolytica* in faeces, cultures or tissue samples has been hampered by several difficulties, particularly in developing countries in which the disease is common. These problems are associated with the traditional microscopical examination of fresh samples and those stained with iron haematoxylin (IH) (Amaral & Mayrink, 1957), or Wheatley's trichrome. The morphological preservation of trophozoites and cyst forms, their frequency and the presence of similar structures in the sample can prevent correct identification of the organism. This study describes an immunocytochemical assay to detect *E. histolytica* at different stages of its life cycle and in different samples. The immunoperoxidase (IP) technique, which has a wide application in the diagnosis of infectious diseases, mainly due to its high sensitivity and specificity (Polak & Van Noorden, 1983), was used to provide a fast and reliable assay for the identification of *E. histolytica* in positive stools, rectal exudates, cultures and tissue autopsy specimens.

Materials and Methods

Sources of samples. Stool samples were obtained from patients with chronic diarrhoea and dysentery with amoebiasis (positive IH staining of *E. histolytica*). Rectal exudates were obtained from ulcerative lesions of the rectum or sigmoid colon of children with amoebic dysentery. Liver and intestinal autopsies from patients with liver abscesses were fixed in 10% buffered formalin for 24 h and embedded in paraffin. Caecal exudate samples were obtained from Sprague-Dawley rats infected with 5×10^6 to $1{\cdot}5 \times 10^6$ *E. histolytica* trophozoites 8-15 d previously. In addition, *E. histolytica* trophozoites of the polyxenic strains HV71:UCV, HV74:UCV, HV75:UCV, HV78:UCV, HV81:UCV, and HV83:UCV cultivated using the Boeck & Drbohlav (1925) method as modified by De La Torre *et al.* (1970), and the axenic strain NIH:200 cultivated as described by Diamond *et al.* (1978), were used. Stool samples negative for *E. histolytica* but positive for other parasites (*E. coli*, *Iodamoeba buetschlii*, *Giardia lamblia*, *Chilomastix mesnili*, *Trichomonas hominis*, *Blastocystis hominis* and *Acanthamoeba* spp.) were tested for possible cross-reactions or false positive results. Also, as negative controls, leucocytes, endothelial cells and stools from healthy children were used.

Microscopic methods. To detect the presence of parasites, fresh stool samples prepared in saline and Lugol's solution were examined. The stools were then preserved in Schaudinn's medium for additional confirmation of trophozoites and cysts of *E. histolytica* by IH staining (Amaral & Mayrink, 1957).

Antisera. Human anti-*E. histolytica* immunoglobulin was prepared by mixing a pool of sera obtained from patients with known titres of antibody against *E. histolytica*, determined by counterimmunoelectrophoresis. IgG was purified using a DEAE cellulose column (O'Shea & Feria-Velasco, 1974).

Immunoperoxidase staining. For IP procedures, the stool smears were fixed in methanol for 3 min prior to immunostaining. 5 μm sections of tissue samples were collected on slides using glycerinated-albumin as adhesive. For histological analysis the tissue sections were stained with haematoxylin and eosin (H&E).

An indirect IP technique was carried out according to the method of Nakane (1975), modified in the following manner. (1) Blocking of endogenous peroxidase with 3% H_2O_2 in methanol, 20 min; (2) background blocking with normal goat serum diluted 1:30, 20 min.; (3) primary antiserum, anti-*E. histolytica* diluted 1:1000, 1-2 h; (4) washing in phosphate-buffered saline, pH 7·2 (PBS), 5 min; (5) goat anti-human IgG conjugated to peroxidase diluted 1:100, 1 h; (6) PBS, 5 min; (7) developing 10 min with 90 μM H_2O_2 and 3-amino-9-ethyl-carbazole (final concentration 0·88 mM), dissolved in 50 mM N,N-dimethylformamide in 0·1 M acetate buffer, pH 5·2; (8) rinsing in water; (9) mounting in glycerine-gelatine. Tissue samples were counterstained with Mayer's haematoxylin. All the incubations were at room temperature.

To evaluate the sensitivity of the IP staining method, the procedure was carried out using trophozoites of the HV71:UCV strain which were serially diluted from an initial concentration of 32 500 protozoa/slide to a final concentration of 3 protozoa/slide.

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Results

The Table shows the results obtained using either the IP or IH staining. The IP method allowed the detection of *E. histolytica* in almost all the positive samples (Fig. 1). Trophozoites were strongly stained in all the cultivated strains tested, and in all phases of the life cycle of the amoeba. In addition, both intact and damaged cysts could be detected in all positive stool samples (Fig. 2). No reaction was observed with leucocytes, endothelial cells, or with cyst or vegetative forms of any other protozoan tested, including *E. coli* and the free-living amoeba *Acanthamoeba* sp. The IP method gave positive results at the dilution equivalent to 3 trophozoites per slide. No difference in sensitivity was found between the IP test and IH staining (Table 1).

Discussion

The visual detection of *E. histolytica* in faeces, tissue or cultures is often difficult, because samples may contain other parasites, structures or cells with similar size, morphology or appearance to *E. histolytica* (see SHEEHAN *et al.*, 1979). Erythrophagocytosis must be observed to confirm the pathogenicity of the protozoan (TRISSL *et al.*, 1978), and the nuclear characteristics of the amoeba, which are important for identification by IH staining, are not constant in trophozoites from either cultures or rectal exudates.

The IP method has several advantages over direct microscopical examination, and also other immunological assays. In contrast to ELISA (ROOT *et al.*, 1978; GRUNDY, 1982), this method was shown to be sensitive (98·6% of samples positive for *E. histolytica* were identified) and specific (no nonspecific reaction was observed). Also, all forms of the life cycle were stained, whereas ELISA is less useful for the detection of cyst forms (GRUNDY, 1982). ELISA based upon nitrocellulose supports can also give nonspecific reactions (ROOT *et al.*, 1978). The technique is easier to perform and requires less equipment than ELISA; it is also convenient, as fixed samples can be stored for at least one month before immunostaining.

The technique of immunostaining with peroxidase detects superficial and intracellular antigens of *E. histolytica* trophozoites (FERIA-VELASCO & O'SHEA, 1974; AUSTT KETTIS & UTTER, 1984); this report demonstrates its usefulness in clinical diagnosis. Eventually, the use of monoclonal antibodies should be evaluated to facilitate the application of this test on a large scale.

These preliminary results indicate that IP staining is a reliable, sensitive, specific and rapid method to diagnose *E. histolytica* infection. It should now be evaluated using larger groups of patients, with varying degrees of clinically apparent amoebiasis.

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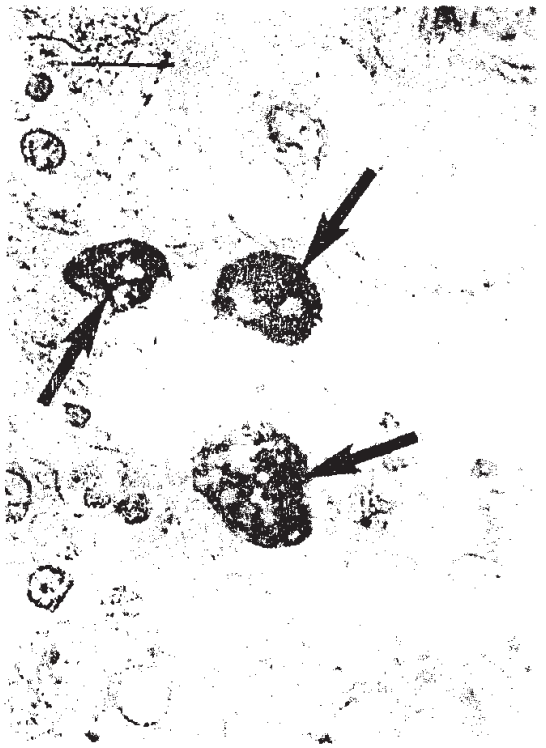

Fig. 1. Immunoperoxidase staining of *E. histolytica* trophozoites (arrows) within hepatic abscess of a patient.
Scale bar = 10 μm.

Fig. 2. Amoebic cyst in stool sample immunostained using a specific anti-*E. histolytica* serum and indirect immunoperoxidase. Scale bar = 20 μm.

Table—Application of immunoperoxidase and iron haematoxylin staining to samples containing a variety of protozoa and other cells

Sources and content of samples	No. of samples	No. positive for *E. histolytica* IP	IH or HE
Positive samples			
Faeces, *E. histolytica*	14	14	13
Human rectal exudates, *E. histolytica*	10	10	10
Rat caecal exudates, *E. histolytica*	8	7	8
Liver and large intestine autopsies (*E. histolytica*)	5	5	5
Cultures, strain HV71:UCV	22	22	18
Cultures, strain HV74:UCV	2	2	2
Cultures, strain HV75:UCV	3	3	3
Cultures, strain HV78:UCV	3	3	3
Cultures, strain HV81:UCV	1	1	1
Cultures, strain HV83:UCV	1	1	1
Cultures, strain NIH:200	5	5	5
Control samples			
Cultures, *Acanthamoeba*	3	—	—
Faeces, *I. buetschlii*	1	—	—
Faeces, *E. coli*	3	—	—
Faeces, *G. lamblia*	3	—	—
Faeces, *T. hominis*	2	—	—
Faeces, *B. hominis*	3	—	—
Faeces, *C. mesnili*	1	—	—
Leucocytes and endothelial cells	11	—	—
Faeces, healthy children	24	—	—
	125	73	69

IP = Immunoperoxidase
IH = Iron haematoxylin
HE = Haematoxylin & eosin
IP vs IH or HE not statistically significant (χ^2: $P>0{\cdot}05$)

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