ENTAMOEBA POLECKI: MORPHOLOGY, IMMUNOLOGY, ANTIGEN STUDY AND CLINIC OF THE FIRST INFECTIONS IN CZECHOSLOVAKIA

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Abstract. Entamoeba polecki. Proc. 1912 was recorded for the first time in Czechoslovakia in two students from Kampuchea. Uninucleated cysts of diameter 12.2–13.7 μm with nuclei diameter 3.2–4.2 μm were found repeatedly in stool samples taken from them. The nucleus accounted in average for 24.3% of the cyst diameter. The isolation and two following subcultivations on Dobbell-Leidlaw medium were achieved, more abundant growth was recorded on medium with pig serum. Stools of both students were positive in serological tests using the E. histolytica antigen. No serious clinical symptoms were observed, both patients were cured successfully by metronidazole and ornidazole. Electroimmunotransfer blots were used to characterize E. polecki as a separate species. The antigenic structure of polymorphically grown E. polecki was compared with the antigenic structure of E. histolytica (occasionally grown HK-9 strain and 3 polymorphically grown strains). Different blot patterns of both species were obtained, but common fractions of 30–40 kDa probably responsible for serological cross-reactions were found.


All human infections were diagnosed by finding uninucleated cysts or trophozoites in the stools. Serological tests with E. histolytica antigen were carried out seldom with either negative results (Masure et al. 1980, Salaki et al. 1979) or non-significant titre (1:64) using the indirect haemagglutination test (Chacin-Bonilla 1983). Using modern biochemical methods Sargeaunt et al. (1980) showed similar zymodemes in E. histolytica strains as in amoebae morphologically identical with E. polecki (zymodemes group I, III, or IV). They agree with other workers who considered the E. polecki as a phase during the life cycle of E. histolytica (Lubinsky 1952, Robinson 1963, Levine 1973).

Two human E. polecki infections in Kampuchean students were discovered for the first time in Czechoslovakia. An electroimmunotransfer blot was used to determine the species E. polecki.

MATERIALS AND METHODS

Examination of stools. Parasitological examination of foreigners after their arrival in Czechoslovakia from tropical countries is obligatory. Using the methotol-sodium-formaldehyde concentration technique, uninucleated cysts of amoeba were found in the stools of two Kampuchean students on November 3rd, 1986 (Fig. 1A). Cysts of Giardia lamblia were also present. On November 7th, 1986 no cysts were observed in the stools of both students. On November 19th, 1986 trophozoites were isolated on Dobbell-Leidlaw medium from stool specimens of both persons (Fig. 1B, C). The isolation of trophozoites was repeated on December 15th, 1986. With the last examination before the treatment (December 29th, 1986) the cysts and trophozoites were found in the stools of only one of the infected.
Serological examinations. The first sample of serum was taken on November 17th, 1985. The second sample on January 5th, 1987. The ELISA, indirect immunofluorescence test (IFAT) and counter immunoelectrophoresis (CIEP) were carried out. An antigen was prepared from axenically grown *E. histolytica* strain HK-9 in Diamond medium.

**E. polecki** antigen study. The antigenic structure of axenically-grown *E. polecki* was compared with the antigenic structure of *E. histolytica* strains (axenically-grown HK-9, and axenically-grown T. PA 35). Serum from a patient with amoebic liver abscess and from a man infected with *E. polecki* were used for an electroimmunotransfer blot (EITB).

The amoebae lysate samples prepared from the trophozoites washed in PBS by repeated freezing-thawing were separated by SDS-polyacrylamide gel electrophoresis according to the Laemmli (1970) method using 5–15% gradient gel. EITB was performed in a home-made apparatus using a buffer containing 25 mM TRIS, 191 mM glycine, 20% methanol and 0.04% SDS (Towbin et al. 1977). The proteins were transferred to nitrocellulose sheet (Schleicher and Schuell 100 ml of BA 85) at 30 V per 2.5 cm thick blotting sandwich for 2 hours with cooling by tap water. The efficiency of transfer was checked by Coomassie blue staining of gels. The immuno-peroxidase staining was carried out according to the following procedures:

1. Blocking of nitrocellulose in 10% horse serum in PBS-0.05% Tween 20 overnight.
2. Washing in PBS-Tween 5 × 2 minutes.
3. Incubation in rabbit hyperimmune or human immune serum with ELISA titre > 1 : 6,400 in PBS-Tween (L) 1 : 100 for 2 hours.
4. Washing as in 2.
5. Incubation in second antibody-peroxidase conjugate — SwAR/Px SwAHoJG/Px (SOL, Prague) respectively in PBS-Tween (1 : 1,000) for 1 hour.
6. Washing as in 2.
7. Developing in a 0.025% solution of 25 mg o-diaminobenzidine tetrahydrochloride (Lachema, Brno) in 100 ml of 0.1 M TRIS HCl buffer pH 7.6 with addition of 30 μl of H₂O₂ (30%) immediately before using.
8. Rins by washing out reagents in distilled water.

Clinical observations. All routine laboratory tests, including estimation of immunoglobulins in serum and circulating immunocomplexes were made. The following procedures were performed: proctoscopy and rectal biopsy (histological examination).

The infections were treated at the beginning with metronidazole at a dosage of 750 mg three times daily. Since side effects (nausesa) appeared on the fourth and fifth day respectively the metronidazole was substituted with ornidazole in a dosage of 500 mg three times daily.

Total duration of the treatment with both drugs was 10 days.

**RESULTS**

Examination of stools. Irregular presence of cysts in the stools of both infected persons was observed during the repeated examinations. Only unicystic cysts were present. The diameter of cysts ranged from 14.2 to 15.7 μm. Nuclei of cysts ranged from the 3.2 to 4.2 μm. The nucleus accounted for 22.5% to 29.5% (24.3% on average) of the cyst diameter.

Two of four isolations were successful in one patient and all of three isolations in the other one. The growth of trophozoites was more abundant in Dobell-Leidlaw medium prepared using pig’s serum. Parasites grew 3 passages only (1 week) in every isolation.

Serological examinations. The level of circulating antibodies measured by three different serological tests is given in Table 1.

**E. polecki** antigen study (Fig. 2A). Identification of the antigenic structure of *E. polecki* stock using human serum from the patient with the amoebic liver abscess (ELISA titre > 1 : 6,400) showed 3 antigenic fractions of 30–40 kD. These fractions seem to be identical with the antigenic fractions in all *E. histolytica* strains tested. Strain HK-9 obtained from the other distinct strains (Fig. 2B). The antigenic structure of *E. polecki* tested by EITB with the serum from the student infected with *E. polecki* demonstrated 2 antigenic fractions in the zone of 30–40 kD. The HK-9 strain presented
Table 1. Circulated antibodies to *E. histolytica* measured by three different serological tests

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serological tests</th>
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<tbody>
<tr>
<td>I.</td>
<td></td>
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<tr>
<td>first serum</td>
<td>IFAT</td>
</tr>
<tr>
<td>second serum (49 days later)</td>
<td>—</td>
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<tr>
<td>1 : 100</td>
<td>positive</td>
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<tr>
<td>II.</td>
<td></td>
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<td>1 : 100</td>
<td>positive</td>
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</table>

**Clinical observations.** At the time when infection with *E. polecki* was discovered, the both infected persons appeared to be healthy. But previously multiple episodes of diarrhea in the students as well as members of their families had been noticed. Erythrocytes sedimentation rate and red blood cells were normal. White blood cells in one patient were normal, the second one had 10,000 W.B.C/mm³ and 11% of eosinophils. IgG was elevated on 21 mg/ml (normal level 7–15 mg/ml) in one of the infected only. No circulated immunocomplexes were estimated. Proctoscopic examination as well as histological examinations of rectal biopsies did not show any pathological changes.

The control examinations of stools immediately after the treatment and three months later were negative for cysts and trophozoites of *E. polecki* and *G. lambia*.

**DISCUSSION**

Having examined many persons from Southeast Asia we discovered uncinculated amoebic cysts quite often. But later, by repeated examinations, cysts with 2–4 nuclei appeared in the stools. In these two infections observed during more than one month, cysts in each sample were uncinculated only. One of the infected persons had had previous contact with pigs in Kampuchea, the second denied it.

Morphological differentiation *E. hartmanni* and *E. polecki* from *E. histolytica* was described in detail by Burrows (1959), Salaki et al. (1979) and De Girolami and Kimber (1983) added their comments on morphological diagnosis. We tried to recognize *E. polecki* as a separate entity using modern biochemical methods for characterization of its antigen (Fig. 2A) presented the antigenic architecture of *E. polecki* and 4 strains of *E. histolytica*. Although differences in the antigenic composition between both species exist, the fractions of 30–40 kD are common both for *E. polecki* and *E. histolytica*. These fractions could probably respond for serological reaction between *E. histolytica* antigen and anti *E. polecki* antibodies. The serological reactivity was also between serum from the *E. polecki* infected man and antigen *E. histolytica* HK-9 strain (Fig. 2B).

Similar results were achieved by De Carli (1983) who studied the antigenic composition of 4 *Trichomonas* species. Common antigens exist also in 4 species of malaria parasite in man.

Up to now we cannot say unambiguously if *E. polecki* is a separate species. Further studies with axenically growing *E. polecki* strains, a greater number of infected persons and hyperimmune sera from experimental animals could elucidate one of the interesting questions connected with mysterious organism *E. histolytica*. Our results indicate that those authors who consider *E. polecki* as a separate species might be right.

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**ΕΝΤΑΜΟΕΒΑ ΡΟΛΕΚΙ: ΜΟΡΦΟΛΟΓΙΑ, ΙΜΜΥΝΟΛΟΓΙΑ, ΙΣΤΟΧΟΜΙΟΛΟΓΙΑ ΚΑΙ ΕΞΩΤΕΡΙΚΗ ΠΡΩΤΗ ΙΝФΕΚΣΗ ΝΑ ΤΗΣ ΤΕΡΡΙТОΡΙΑΣ ΚΕΧΟΧΟΛΑΒΚΙΝ**

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**Ρεζίσεις.** Ενταμοεβά polecki Πρωτεκττάς, 1912 βασικά ιονοβολώνοντας σε διαφόρο ηλεκτρολυτικά ιόντα ινθετρότο σε 1.4–1.5, μικρούς με έχοντα ιόντα σε 3.2–3.2 μικρούς.
Ядра занимали в среднем 24,3 % от диаметра цисты. Амёбы удалось изолировать и провести две последующие субклонировки в среде Dobell-Leidlaw, более выраженный рост наблюдался в среде с добавкой сыворотки крови. В течение инфекции не наблюдали серьёзных клинических симптомов, обнаруживали мегазоносом и ориндозом. Для характеристик E. polecki как отдельного вида использовали электронную микроскопию и флуоресцентную антителную реакцию. Антителная структура полисахаридной капсулы E. polecki сравнивали с тем же структурой у E. histolytica (аэробиическая культура лиофилизованных клеток). Были получены различные картины антител E. polecki и E. histolytica, но была найдена фракция 30-40 kDa общая для обоих видов, которая отвечает за перекрёстные серологические реакции.

REFERENCES


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