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A Redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) Separating It from *Entamoeba dispar* Brumpt, 1925[1]

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ABSTRACT. Explaining the low incidence of invasive disease (10%) in humans infected with *Entamoeba histolytica* has occupied the attention of generations of both clinical and nonclinical investigators. One possible explanation would be the existence of two morphologically identical species—one an invasive pathogen, the other noninvasive. This was first proposed by Brumpt in 1925, but his explanation was virtually ignored until 1978 when the first of several publications appeared suggesting that *E. histolytica* did indeed consist of two species. We have reexamined Brumpt's claim in light of recent biochemical, immunological and genetic studies and conclude that the data derived from these investigations provide unequivocal evidence supporting his hypothesis. With this in mind, we redescribe the invasive parasite retaining the name *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911), and set it apart from the noninvasive parasite described by Brumpt, *Entamoeba dispar* Brumpt, 1925.

Supplementary key words. Amebiasis, antibodies, diagnosis, genes, isoenzyme analysis, speciation.

STUDENTS of *Entamoeba histolytica* and the diseases it causes have for generations faced one vexing question overriding all others: how can we account for the well documented fact that only a few of those humans infected with the parasite develop invasive disease? A modern estimate is that less than 10% (36 million) of those infected with *E. histolytica* (480 million) develop clinical symptoms, of which at least 40,000 die annually [55]. Three major hypotheses have been advanced to explain this phenomenon and can be summarized as follows: 1) *E. histolytica* is a single pathogenic species which in all human hosts produces intestinal lesions that may or may not give rise to recognizable clinical symptoms; 2) *E. histolytica* is normally a commensal residing in the human colon which on occasion, for reasons poorly understood, converts into an invasive pathogen; 3) *E. histolytica* is comprised of two morphologically identical species, one an invasive pathogen exhibiting varying degrees of virulence and the other a noninvasive pathogen having the capacity of producing at most superficial erosion of the colonic mucosa. Recent studies have provided evidence that the third hypothesis is the correct one.

That humans can be infected by two morphologically identical species of *Entamoeba* producing quadrinucleate cysts measuring 10 μm or greater in diameter was proposed first by Brumpt in 1925 [5]. He distinguished the two species on the basis of their pathogenicity in humans and in experimentally infected kittens. The invasive pathogen was identified as *Entamoeba dysenteriae* (Councilman and Lafleur) Craig, 1905, which according to Dobell [10] is a synonym for *Entamoeba histolytica* Schaudinn, 1903 (Emend. Walker, 1911). For the noninvasive ameba, Brumpt created the name *Entamoeba dispar*. Simic [40–42] provided additional convincing evidence for the existence of the two species. He serially passed several amebal isolates in healthy volunteers and kittens, a very sensitive model, and between kittens and humans. Neither the kittens nor humans developed symptoms attributable to the presence of the amebae.

Because Brumpt was unable to distinguish morphologically between his two proposed species, and because there was a growing body of evidence obtained from experimental human and animal studies that amebae obtained from symptomless carriers could produce disease [19, 24, 25, 57], his explanation gained little support. It regained favor only after Sargeaunt and

[1] This paper is dedicated to the memory of the late Ralph A. Neal who made major contributions to the study of these organisms.

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colleagues reported distinguishing two groups of *E. histolytica* on the basis of isoenzyme typing—one group isolated from asymptomatic individuals, the other from symptomatic patients [38].

In this paper we review the biochemical, immunological and genetic evidence for separating *E. histolytica* into two species and redescribe *E. histolytica* Schaudinn, 1903 (Emended Walker, 1911) [39, 56], the invasive pathogen, setting it apart from the noninvasive *E. dispar* Brumpt, 1925 [5].

MATERIALS AND METHODS

All the materials and methods used in obtaining the data to be presented have been published in the articles cited in Results and Discussion. The morphological description of *E. histolytica* is based in part on the personal experiences of one of us (LSD), who has examined stools and/or rectal biopsies from hundreds of cases of amebiasis, and in part on descriptions by Dobell [10] and Brumpt [5]. Unfortunately, Schaudinn's description of the ameba [39] is considered to be inadequate by all authorities and is not used. In interpreting Dobell's description of *E. histolytica* [10], one of the best available, it must be kept in mind that he did not recognize *E. hartmanni* von Prowazek, 1912 as a species distinct from *E. histolytica* and thus the measurements given by Dobell encompass those of that smaller ameba.

RESULTS AND DISCUSSION

The evidence for two species. *Biochemical evidence.* Electrophoretic isoenzyme analysis of several thousand isolates of *E. histolytica* using four glycolytic isoenzymes [hexokinase, glucosephosphate isomerase, phosphoglucomutase and L-malate NADP+ oxidoreductase (malic enzyme)] led to their division into several zymodemes. More importantly, these zymodemes fall into two groups. One, designated "pathogenic" (P), consisted of amebal isolates the vast majority of which were from patients with invasive disease. The other, designated "nonpathogenic" (NP), consisted exclusively of isolates from asymptomatic carriers [37, 38]. In the most recent report [3], the profiles of 17 P and 19 NP isolates were examined using 12 isoenzymes, including the four mentioned above, to determine their taxonomic relationship. Distance analysis clearly demonstrated that P and NP isolates fall into two genetically distinct groups and several enzymes were found to distinguish unambiguously between the two.

Immunological evidence. Since monoclonal antibodies distinguishing the two were first reported in 1988 [43], several additional studies have demonstrated immunological differences between P and NP isolates of *E. histolytica*:

1. Monoclonal antibodies against the galactose-specific adherence lectin revealed that four of six epitopes on P isolates were not present on NP forms [32].
2. A 96-kDa surface antigen present on three xenically and four axenically cultivated pathogenic isolates was identified and characterized. The antigen was absent or at least present in lesser quantity on six xenically cultivated NP isolates [51, 53].
3. Antibodies against a 29-kDa antigen detected on the surface of P isolates differentiated between P and NP isolates [34, 52]. A 30-kDa antigen was detected by a monoclonal antibody in 42 isolates exhibiting P isoenzyme patterns, and was absent in 14 isolates having NP patterns [45]. Subsequent analysis revealed that these 29-kDa and 30-kDa antigens are the same molecule.
4. A 30-kDa surface antigen was identified by Western blots with human serum in 15 P isolates and was absent in 15 NP isolates. This particular antigen of *E. histolytica* differed from the others described in that it was not detected in amebae cultivated axenically, despite the amebae having been originally isolated from patients with invasive disease [2].
5. A monoclonal antibody against electron-dense granules (EDG) related to collagenolytic activity in *E. histolytica* was described which reacted with P but not NP isolates. This suggested that the EDG antigen may be differentially expressed in P and NP isolates [31].
6. A monoclonal antibody detecting a 81/84-kDa intracellular antigen was described that reacted in an indirect immunofluorescence assay with 44 P isolates but not with 44 NP isolates [16].

Genetic evidence. The most convincing evidence supporting the two species concept comes from a number of studies conducted at the DNA level:

1. In the first of these, two DNA probes, P145 from a P isolate and B133 from a NP isolate, were cloned from highly repetitive elements on the amebal extrachromosomal circular DNA molecules. These hybridized selectively to DNA of isolates having P and NP isoenzyme patterns, respectively [4, 12].
2. cDNA probes for related single-copy genes, one specific for P isolates (cEH-P1) and the other for NP isolates (cEH-NP1), were identified [49]. In a separate study, a gene (M17) encoding a 125-kDa surface antigen was detected and characterized from a P isolate and was also present in NP isolates [11]. The P genes isolated by the two groups proved to encode the same molecule, as did the two NP genes. However, sequence analysis and comparison revealed a 12% divergence in deduced amino acid sequence between the P and NP genes. Using PCR amplification of gene fragments followed by restriction digestion the two amebal forms could also be distinguished [11, 47], and in a study of amebal isolates from 48 human infections a strict correlation was found between the classification of P and NP isolates by isoenzyme pattern, restriction pattern, and clinical status [47].
3. cDNAs encoding the 29-kDa/30-kDa antigen were isolated from P and NP isolates, and the nucleotide sequences compared (approximately 400 bp). An inferred amino acid sequence difference of 4.5% between the two forms was found [44]. Again, restriction digestion of PCR amplified gene fragments could distinguish the two [44] and form-specific PCR primers were also designed [46].
4. In a study of homologous cysteine proteinase genes, differences in structure and expression were found between P and NP isolates. A 17% divergence in the predicted amino acid sequence was found. The organization of the genes as revealed by Southern blotting was conserved within each group, but none of the genes studied were found in both P and NP isolates [50].
5. Comparison of cDNA sequences for the single copy iron-containing superoxide dismutase genes of P and NP isolates revealed a 5% nucleotide divergence and a single amino acid change between the two. A consistent restriction fragment size difference between isolates of the two forms was revealed by probing Southern blots of restriction enzyme digested total DNA with this gene [48].
6. The genomic organization of the actin genes appears to differ between the two forms [49].
7. Using PCR amplification and restriction digestion the highly conserved small subunit ribosomal RNA (rRNA) genes of 18 P and 13 NP isolates were compared. The two groups were found to give quite distinct patterns, but within each group all patterns were identical. On partial sequencing of genes from three P and three NP isolates a genetic distance of 2.2% was found between the two forms, a difference greater than that occurring between humans and mice [7]. Southern blots of restriction enzyme digested total DNA from several P and NP isolates were analyzed

using recombinant ribosomal DNA probes, revealing consistent pattern differences between the two forms [9, 33].

The evidence presented in many of the publications cited above for the immunological and DNA distinctiveness of P and NP isolates was derived from molecules potentially involved in pathogenesis. Such sequence differences could be interpreted as being directly linked to the difference in virulence between the two forms. However, rRNA genes at least are immune to such concerns. The most reasonable conclusion to be drawn from the data presented is that *E. histolytica* comprises two distinct genetic entities and the degree of divergence supports the two-species concept of Brumpt [5].

The evidence for a single species. The reports opposing the two-species view support the second hypothesis, that of a commensal *E. histolytica* capable of conversion into an invasive pathogen. Conversion of the isoenzyme patterns of certain cloned and uncloned NP isolates to those of the P form was noted during attempts to axenize NP amebae [1, 27, 28]. Axenization had not been achieved when the change occurred. Reversion back to the NP pattern was accomplished by reassociating the amebae with their original flora. Furthermore, in two of the studies it was reported that conversion of the isoenzyme patterns was accompanied by a parallel alteration in virulence from avirulent to virulent [27, 28].

In contrast, evidence that the isoenzyme patterns of *E. histolytica* are stable properties is provided convincingly in three other reports. In one, no alteration in the isoenzyme pattern of any amebal isolate was observed up to nine years after primary isolation, nor had any mutation been found to occur in culture during this same period [36]. In a second report, varying the bacteria flora of several isolates did not lead to alteration of the isoenzyme pattern [37]. In the third report the authors of an 18-month in-depth study set out purposely to convert the isoenzyme patterns of five amebal isolates exhibiting NP patterns, including two isolates used in the conversion reports cited above. All of the variables proposed to be involved in inducing conversion, both individually and in combination, were examined. All of the attempts failed—no conversion of the isoenzyme patterns could be detected. On the basis of their own and earlier studies the authors interpreted the reports of isoenzyme conversion as artifactual [8].

Hybridization patterns obtained using the repetitive DNA probes P145 and B133 with the original and converted forms of two NP isolates reflected the respective zymodemes of the amebae [12]. Other reports have confirmed that these converted NP strains resemble typical P amebae at the immunological and DNA levels [4, 7, 9]. To account at the genetic level for the phenomenon of conversion it was speculated that "all *E. histolytica* strains contain copies of the same sequences in their genomes, but changes in certain conditions of growth may cause the amebae to amplify different elements and express modified amebic components and behaviors which would remain undetected under other culture conditions of growth" [12]. In a later report using PCR techniques evidence was presented for the existence of one or a few tandemly repetitive P145 elements characteristic of P isolates in a NP isolate. Technical difficulties reportedly prevented finding copies of the NP-specific B133 repetitive elements in P amebae [29].

In view of the above findings a low copy number P rRNA gene was sought in a NP isolate, but under conditions where the PCR technique was capable of detecting one P in 10,000 NP amebae the presence of even a single P rRNA gene in the NP isolate could not be detected. Thus, if there was a P gene in the NP isolate it must have been present in a ratio of less than 1 in 10,000. Since the estimated number of rRNA genes per cell is 200–400, it can be inferred that there is less than one P gene in each NP cell, and, since some of the strains studied were cloned lines, it can be stated that P ribosomal RNA genes are not present in the NP genome [7].

Finally, given the extensive genetic divergence mentioned above, an explanation for conversion would require a mechanism for an alteration and rearrangement of amebal genes unprecedented in scope in eukaryotic genetics and a molecular machinery unique to *E. histolytica*, as conversion has not been reported during axenization of other *Entamoeba*.

In view of the overwhelming body of evidence supporting the concept that *E. histolytica* is a complex of two species we now redescribe *E. histolytica* Schaudinn, 1903 (Emended Walker, 1911), separating it from *E. dispar* Brumpt, 1925.

Entamoeba histolytica Schaudinn, 1903 (Emended Walker, 1911)

Morphological characters in vivo. Trophozoites ranging in size from 20 to 40 μm in diameter. Locomotion rapid, gliding, by means of a single well defined pseudopodium, often extended explosively, without conspicuous differentiation between ecto- and endoplasm. Cytoplasm with a single nucleus, rarely up to four; often with ingested red blood cells, sometimes with leukocytes, or bacteria; rich in glycogen; with ribosomes arranged in helices which aggregate to form characteristically shaped elongate bars with rounded ends (= chromatoid bodies). Without classical mitochondria, rough endoplasmic reticulum, or golgi apparatus. Nucleus vesicular, spherical, measuring 4 to 7 μm in diameter, consisting of a delicate achromatic membrane lined usually by a single layer of small chromatin granules, uniform in size, in contact or very close to each other. With a small spherical karyosome (0.5 μm in diameter), often centrally located, surrounded by an achromatic capsule-like structure.

Cysts spherical, measuring 10 to 16 μm in diameter, four nuclei when mature, rarely up to eight; glycogen in a distinct vacuole in the immature cyst, becoming more diffuse as the cyst matures; often with chromatoid bodies in the immature cyst, disappearing as the cyst matures. Nuclei with morphology similar to those of trophozoites.

Geographic distribution. Worldwide.

Hosts. Humans, several nonhuman primates, cats, dogs and rats are well documented hosts. Reports of infection in other mammals must be viewed with caution unless evidence of tissue invasion has been provided, and/or the parasite has been isolated and subjected to isoenzyme or other discriminatory analysis. Levine [20] provides the best overall review of reported hosts.

Characters separating *E. histolytica* from *E. dispar*. *Biochemical characters.* Can be discriminated from *E. dispar* on the basis of the migration of any one of six isoenzymes [3, 37]: hexokinase EC 2.7.1.1, phosphoglucomutase EC 2.7.5.1, aldolase EC 4.1.2.13, acetyl-glucosaminidase EC 3.2.1.30, peptidase and NADP-diaphorase. Glucosephosphate isomerase EC 5.3.1.9 does not discriminate the two on starch gels, but does on polyacrylamide gels [22, 26] and cellulose acetate [30].

Immunological characters. Can be separated from *E. dispar* by monoclonal antibodies against several proteins: the Gal/GalNAc adherence lectin [32], the 96-kDa antigen [53], the 29-kDa/30-kDa antigen [34, 45, 52], the EDG antigen [31], the 81/84-kDa antigen [16] and an uncharacterized antigen [4, 43].

Genetic characters. Can be distinguished from *E. dispar* by: **1**) dot hybridization with probe P145 [12]; **2**) fragment pattern comparison of Southern-blotted restriction-digested genomic DNA probed with the actin [49], cysteine proteinase [50], 125-kDa antigen [49], superoxide dismutase [48], or ribosomal [9, 33] genes; **3**) restriction enzyme digestion of PCR amplification products of the small subunit ribosomal RNA [7], 29-kDa/30-

kDa antigen [44] or 125-kDa antigen [11, 47] genes; **4)** PCR amplification of gene fragments with oligonucleotides specific for the small subunit ribosomal RNA [7] or 29-kDa/30-kDa antigen [46] genes.

Clinical features. *Entamoeba histolytica* is a pathogenic species displaying varying degrees of virulence and capable of invading a wide variety of tissues in the host including those of the colon and liver, and more rarely lung, skin, urogenital tract, brain, and spleen. This invasive feature separates it from *E. dispar* which, though it may be a pathogen producing erosion of the colonic mucosa, has not been identified with tissue invasion.

Remarks. It should be stressed that *E. dispar* and 'nonpathogenic (NP) *E. histolytica*' are synonymous. However, applying the term nonpathogenic to *E. dispar* may be inaccurate. The parasite appears capable of inducing focal intestinal lesions in experimental animals such as kittens, gerbils and guinea pigs [5, 6, 42, 54], and thus would be, by definition, a pathogen. The possibility that *E. dispar* can also induce pathological changes in the human colon, although not necessarily invasive lesions, cannot be excluded [15, 23] and deserves further study.

Cysts of *E. histolytica* (= P isolates) have been found in the stool of asymptomatic cyst passers free of *E. dispar* [13, 14, 26] and in patients with amebic liver abscess but who are free of intestinal symptoms [17]. Therefore, asymptomatic cyst passers must not be assumed to carry *E. dispar*. Likewise, a positive serological test need not indicate *E. histolytica* infection since up to 20% of *E. dispar* infections may lead to seropositivity [18, 21, 35].

The existence of two species within what was previously called *E. histolytica* has profound consequences for interpretation of epidemiological data and the older literature, for clinical evaluation of carriers and for estimating the proportion of symptomatic infections. The numerous methods now available for distinguishing *E. histolytica* from *E. dispar* should allow more accurate diagnosis and data collection in the future.

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