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Entamoeba histolytica: The serine-rich gene polymorphism-based genetic variability of clinical isolates from Georgia

Sophia Simonishvili [a], Shota Tsanava [a], Ketevan Sanadze [a], Rusudan Chlikadze [a], Anna Miskalishvili [a], Nino Lomkatsi [a], Paata Imnadze [a], William A. Petri Jr. [b], Nino Trapaidze [a,*]

[a] *National Center for Disease Control and Medical Statistics of Georgia, 9 Asatiani Street, Tbilisi 380077, Georgia*
[b] *Departments of Internal Medicine, Microbiology and Pathology, University of Virginia, Charlottesville, VA 22908-1340, USA*

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Abstract

It is generally accepted that a majority of individuals infected by *Entamoeba histolytica* do not develop symptomatic disease. However, the parasite and the host factors contributing to the development of the disease, remain undetermined. It is also unclear why certain individuals develop extra-intestinal amebiasis without exhibiting apparent intestinal symptoms. An outbreak of amebic liver abscess in Tbilisi, Georgia in 1998–1999 suggested that the causative *E. histolytica* strain had an unusual propensity for extra-intestinal spread. To correlate the genetic differences with pathogenic potential of the parasite, we have examined the SREHP gene polymorphisms among Georgian *E. histolytica* isolates. Comparison of polymorphic patterns revealed the presence of several different genotypes of *E. histolytica*, thus preventing an association of a single genotype with hepatic disease, but supporting the previous finding of extensive genetic diversity among *E. histolytica* isolates from the same geographic origin.

Index Descriptors and Abbreviations: *Entamoeba histolytica*; *Entamoeba dispar*; Protozoa; Parasite; Amebiasis; Polymorphic DNA; Tandem repeats; Genotypes; Endemic population; SREHP, serine-rich *E. histolytica* protein; SSG, strain specific gene; PCR, polymerase chain reaction

1. Introduction

Amoebiasis, the disease caused by pathogenic parasite *Entamoeba histolytica*, is most common in developing countries. Infection, acquired by oral ingestion of cyst-containing food or water, resulting in development of amebic colitis and liver abscess, is second after malaria as a protozoan cause of death (Petri and Singh, 1999). The vulnerability of the developed world to epidemics of this disease was clearly demonstrated by a large waterborne outbreak in Tbilisi, Republic of Georgia in 1998–1999 that was characterized by extremely high number of liver abscess cases (Barwick et al., 2002). Although the number of registered amoebiasis cases has gradually decreased after the outbreak in Tbilisi (136 cases in 2002 and 33 in 2003), the propensity to cause liver abscess has remained (NCDC, 2004).

An earlier outbreak of the disease in Tbilisi in 1927 consisted of 800 cases, 80% of which were intestinal, 3% extraintestinal, and 17% mixed (Svanidze, 1959). Since amoebiasis was almost eradicated later, with only 1–3 cases registered annually in the 1960s and 1970s, it was excluded from the list of reportable diseases in 1971. The apparent potency of *E. histolytica* to cause liver abscess in the most recent outbreak has led us to study and characterize the "Georgian version" of the parasite with the aim to elucidate the molecular basis of virulence.

It is estimated that annually 40–50 million individuals are infected worldwide, resulting in 100,000 deaths

* Corresponding author. Fax: +995 32 94 1085.
E-mail address: trapaidze@yahoo.com (N. Trapaidze).

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(WHO, 1997). It was generally accepted that a majority of individuals infected with *E. histolytica* did not develop symptomatic disease. However, the finding that the parasite known as *E. histolytica* consisted of two morphologically identical but genetically different species, has made a major impact on further developments in the field. Although, the difference between *E. histolytica* and *Entamoeba dispar* was first suspected in 1925, reliable evidence delineating *E. histolytica* into these two distinct invasive, pathogenic *E. histolytica* and non-invasive, nonpathogenic *E. dispar*, was only accepted relatively recently (Diamond and Clark, 1993; Tannich et al., 1989). Apparently, 90% of the infections attributed previously to *E. histolytica* were actually *E. dispar*, known to be responsible for nonpathogenic colonization of the intestine and only 10% were *E. histolytica* (Haque et al., 1999; Jackson et al., 1985). Moreover, many *E. histolytica* sensu strico infections also remain asymptomatic and are spontaneously lost, while only a small proportion progress to symptomatic intestinal or extraintestinal disease (Gathiram and Jackson, 1987; Haque et al., 1997).

These observations, suggesting that organisms producing the invasive, symptomatic disease might be genetically different from those producing the asymptomatic infections only, give rise to a whole range of questions, such as how certain groups of infected individuals develop extraintestinal amebiasis without exhibiting apparent intestinal symptoms. The reported high degree of heterogeneity in virulence and inter-strain variations possibly determining the ability of parasite to produce liver abscesses have reinforced efforts through molecular epidemiological studies to determine whether some subgroups of *E. histolytica* are more likely than others to cause invasive disease. The polymorphism of *E. histolytica* strains within and between endemic areas has been demonstrated recently in different countries (Ayeh-Kumi et al., 2001; Ghosh et al., 2000). Interestingly, 34 unique serine-rich *E. histolytica* protein (SREHP) patterns were observed among 54 isolates from children in the Mirpur region of Dhaka, Bangladesh. There the SREHP genotypes of clinical isolates from patients with liver abscesses were distinct from those of clinical isolates from patients with colitis and dysentery, thus suggesting an existence of some association between the SREHP genotypes and clinical presentation (Ayeh-Kumi et al., 2001).

As mentioned above, a significant number of cases of intestinal amebiasis (1330 in 1998 and 692 in 1999) have been registered in Tbilisi, the capital of Georgia, with 404 cases of amebic liver abscess. Investigation of this outbreak in Georgia has indicated that endemic strains might possess certain unique characteristics, most notably an unusual potency to cause liver abscess. Thus, it is of great importance to elucidate the genetic and biochemical differences between these and other known strains of this pathogen. The Gal/GalNAc lectin, a cell surface molecules engaged in recognition and adhesion to distinct receptors on host cells, was the first candidate gene to be investigated. However, sequence analysis of its gene did not show significant differences that could contribute to such an unusual virulence of these strains (Beck et al., 2002). To elucidate further the correlation and relationship between genetic differences and pathogenic potential of *Entamoeba*, here we have examined the serine-rich gene polymorphisms among symptomatic and asymptomatic clinical isolates of *E. histolytica* collected in Georgia. Comparison of polymorphism patterns has revealed the presence of several different types of *E. histolytica*, reflected in SREHP gene polymorphism and thus supporting the previous finding of extensive genetic diversity among *E. histolytica* isolates from the same geographic origin.

2. Materials and methods

2.1. Stool specimens

Stool samples were collected from patients with diarrhea and asymptomatic individuals involved in the study. Specimens were examined by microscopy and "*E. histolytica II*" stool antigen detection kit (Techlab). Stool-derived DNA was tested for the presence of the small subunit rRNA gene. Stool samples negative by microscopy, antigen detection kit and rRNA gene, or containing only *E. dispar* were used as negative controls. DNA isolated from axenic cultures of *E. histolytica* HM1:IMSS and *E. dispar* strains were used as positive and negative controls for PCR amplifications.

2.2. Culture development and maintenance

Microscopy-positive stool specimens collected from symptomatic patients were used for developing parasite cultures. Entamoeba cultures were isolated and maintained in Robinson's medium.

2.3. Isolation of DNA from stool samples and cultures

Stool samples (0.2 g) containing trophozoites and cysts of amebae or 1×10^6 cultured parasites, were used for preparation of DNA using the CTAB method, described elsewhere (Zaki and Clark, 2001). Extracted DNA was further purified by passing through the CHROMA SPIN-1000 Columns (Clontech).

2.4. Nested PCR amplification of SREHP gene

PCR amplification reactions were carried out as described previously (Ayeh-Kumi et al., 2001): primers, SREHP-5 and SREHP-3, amplifying the 549 bp