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DIFFERENTIATION OF *ENTAMOEBA HISTOLYTICA/ENTAMOEBA DISPAR* BY PCR AND THEIR CORRELATION WITH HUMORAL AND CELLULAR IMMUNITY IN INDIVIDUALS WITH CLINICAL VARIANTS OF AMOEBIASIS

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Abstract. To correlate a particular state of immunity with *Entamoeba* spp., we used colorimetric PCR to differentiate *E. histolytica* from *E. dispar* in individuals with amoebiasis and to associate its presence with the clinical profile, including humoral and cellular immune responses to *E. histolytica*. Our results showed high levels of antibody in acute amoebiasis and elevation of IL-4 production, a cytokine related to Th2 profile, associated with *E. histolytica.* In chronic amoebiasis, even with anti-*E. histolytica* seropositivity, intestinal symptoms were associated with *E. dispar* in all the cases, without differences in level of antibodies, BTI, CD4+/CD8+ ratio, INF-γ, and IL-4. Among asymptomatic carriers, *E. dispar* was more frequently found; however, identification of *E. histolytica* in two asymptomatic carriers associated with high levels of INF-γ, a cytokine related to Th1 profile, demonstrate the importance of making specific diagnosis of *Entamoeba* spp., to establish the clinical and epidemiological behavior in both intestinal and extra-intestinal amoebiasis.

INTRODUCTION

Amoebiasis continues to be a significant world-wide health problem. It has been estimated that 500 million individuals are infected with the causal agent *Entamoeba histolytica* and of these, 40 million would develop invasive disease, causing at least 100,000 deaths a year, most of these are secondary to the extra-intestinal complications like hepatic abscess.[1,2]

In accordance with epidemic studies carried out in different parts of the world, the seroprevalence found in some communities of developing countries ranges from 5% to 55%.[3–7] In 1988, the National Serologic Survey in México showed a seroprevalence of 8.41% for the whole country, highlighting the State of Puebla with 14.98%.[8]

The epidemiological analysis of the relationship between the prevalence of infected individuals and the number of cases with invasive amoebiasis made by E. Brumpt in 1925,[9] and confirmed by Walsh in 1986,[1] showed that the disease is present in approximately 10% of people with cysts of *E. histolytica* in feces. This fact suggested the existence of two species of *E. histolytica* to explain the epidemic behavior of amoebiasis in the world.[10,11]

Studies during the last 30 years have confirmed the existence of two morphologically indistinguishable species of *Entamoeba*. *E. histolytica*, the pathogenic species and *E. dispar,* the non-pathogenic species. Recent acceptance by the scientific community of these two different species has had an important impact on our understanding of clinical amoebiasis.[2]

Diverse methods exist to differentiate pathogenicity and virulence from *Entamoeba* spp., most of them requiring the isolation and culturing of the microrganism.[12–16] Recently, Aguirre and others reported the amplification by PCR of specific segments of DNA, and the use of specific probes to allow for the differential diagnosis of pathogenic and non-pathogenic *Entamoeba* spp. in feces.[17]

Usually, the identification of specific antibodies against antigens of *E. histolytica* does not correlate with resistance to the infection or with protective immunity.[18] However, detection of seric antibodies is a useful procedure in the diagnosis of invasive extra-intestinal amoebiasis, but not in the intestinal forms, where it is not feasible to distinguish asymptomatic carriers from patients with amoebic dysentery, especially in endemic areas where antibody titer is not enough to diagnose acute intestinal amoebiasis.[19]

Mosmann and Coffman have shown that specific Th1/Th2 cytokine profiles depend on the subpopulation of CD4+ T lymphocytes.[20] These subpopulations participate in the regulation of the immune response induced by infection with *Leishmania* spp. in animal models,[21] and the data has been confirmed with other pathogenic agents.[22–24] During invasive amoebiasis, decrease of CD4+ cells, increase of CD8+ cells, as well as decrease in *in vitro* proliferation of T lymphocytes against amoebic antigens have been described.[25] Also an immunosupressive state has been observed on delayed cutaneous hypersensitivity against amoebic antigens. On the other hand, high levels of Interferon-γ (IFN-γ) (profile Th1) are associated with a higher cytotoxic effect of macrophages against trophozoites of *E. histolytica*.[26,27] Additionally, studies in a murine model suggest that the induction of the Th1/Th2 profile is dependent on specific peptides of *E. histolytica*.[28]

The asymptomatic/symptomatic clinical state in individuals infected with *E. histolytica* could be explained by the combination of cytokines that reflect the Th1/Th2 profile and the recent acceptance of the two different, pathogenic and non-pathogenic species. In the present work we use colorimetric PCR to differentiate *E. histolytica* from *E. dispar*, to establish correlation between the humoral and cellular immune responses in individuals with clinical variants of amoebiasis coming from an urban community of the state of Puebla, México.

MATERIALS AND METHODS

Study population. The study was carried out in an urban community of the municipality of Puebla, located in the southeast region of México, at an altitude of 1,800 meters and surrounded by high volcanoes. Human population is about 2 million; 60% of them living in an appropriate environment for

the transmission and development of the disease. The estimated seroprevalence in the state, according to the 1988 National Serologic Survey, was 14.98%.[8]

Study subjects. Informed consent was obtained from all humans participants and from parents or legal guardians of minors. The study group was composed of 47 individuals. The diagnosis of the clinical variant of amoebiasis was accomplished by a meticulous clinical study together with parasitolological examination.

The cohort of Acute Intestinal Amoebiasis (AIA) (eleven patients), was clinically diagnosed with colic, tenesmus, trophozoites of *Entamoeba* spp., mucus and/or blood in feces, as well as anti-amoebic antibodies by ELISA (positive serology). Thirteen individuals formed the cohort of Chronic Intestinal Amoebiasis (CIA), showing positive serology, presence of cysts of *E. histolytica/dispar,* and symptoms of alternating diarrhea-constipation with manifestations of abdominal distension, colic, and tenesmus. Thirteen subjects formed the cohort of Asymptomatic Carriers (AC) with positive serology, shedding of cysts of *Entamoeba* spp., and absence of clinical evidence of disease. The Control Group (CG) was formed by 10 asymptomatic individuals with absence of parasite cysts in feces verified by PCR. Blood samples were collected and serum fractions stored at –20°C until used.

Parasitological diagnosis. The morphologic identification of the parasite was carried out by coproparasitoscopic analysis employing a direct method in cases of semi-liquid or liquid feces, or by the modified Ritchie's assay in cases of formed feces.[29] Samples containing cysts or trophozoites of *E. histolytica* and/or *E. dispar*, were diluted in PBS, aliquoted, and stored at –20°C until used. The molecular diagnosis was made by Colorimetric PCR as described.[17,30] The DNA from *E. histolytica* HM1-IMSS and from *E. dispar* clone 53[31] was used as a positive control.

Detection of antiamoebic antibodies in human sera by ELISA. This procedure was performed as described,[32] using 10 μg/mL of *E. histolytica* total extract and human sera at a 1:100 dilution. The cut off value for the ELISA test was an O.D of 0.032 ± 0.009.

Isolation of peripheral blood lymphocytes. Lymphocytes were purified from peripheral blood by the Ficoll-Hypaque (Sigma, St. Louis, MO) method.[33] Cell viability was verified by the trypan blue exclusion test. Cells adjusted to 3.6×10^6 in 1.8 mL of RPMI-1640 culture medium were used for cell proliferation, and 2×10^6 cells in 200 μL of diluent for T CD4+/CD8+ determination.

T cell proliferation assays. Lymphocytes (220×10^3) were cultivated in a 96 well plate, stimulated with phytohemaglutinin (PHA) (1:250), and amoebic total extract[28] (100 μg/mL) at 37°C for 5 days. Proliferation of T cells against antigen or PHA was measured by [^{3}H]-thymidine (1 μCi per well, 6.7 Ci/mmol; Amersham) incorporation after 18 hr. Cells were harvested on glass fiber filters, and incorporated radioactivity was measured by liquid scintillation in a Beckman Instrument (LS5801). Blast transformation index (BTI) was obtained according to Savanat, 1973[34]: BTI = log cpm stimulated cells/log cpm control cells.

CD4+/CD8+ ratio. Determination of CD4+/CD8+ ratio was carried out by flow cytometry using Simultest CD4+/CD8+ kit (Becton-Dickinson^{MR}). Briefly, 1×10^6 lymphocytes in 100 μL were mixed with 20 μL of the respective monoclonal antibody, incubated 15 min at room temperature, washed with PBS/BSA, and centrifuged 10 min at 2,000 × *g*. The pellet was resuspended in 2% *p*-formaldehyde and the cell suspension read in a flow cytometer. Reference values for normal individuals ranges from 0.6 to 2.2.

Cytokine assays. For quantitative determination of IL-4 and INF-γ cytokines, ELISA kits (Genzyme Diagnostics, Predicta®) were used. Supernatant from lymphocyte cultures was incubated with mouse monoclonal antibodies against human IL-4 or INF-γ adsorbed on the bottom of 96 well plates, and then 50 μL of the sample diluter were added per 50 μL of sample. Biotinylated IgGs against IL-4 or INF-γ were incubated for 1 hr at room temperature to perform a sandwich ELISA. After 5 washes, 100 μL of peroxidase labeled streptavidin was added and the plate incubated for 15 min at 37°C. After 5 washes, 100 μL of substrate were added, incubated at room temperature (18–24°C) for 20 min, then 100 μL of 1 N H_2SO_4 were added to each well to change the color from blue to yellow. Plates were read in a spectrophotometer at 450 nm.

Statistical analysis. The statistical evaluation was carried out using the Mann-Whitney U-test . Values of $P < 0.05$ were considered statistically significant. The results in Table 2 are presented as ± one standard deviation. The results in Figure 1–3 are presented as mean ± SE.

RESULTS

Study population. Forty-seven individuals were included in a longitudinal and prospective study (cohorts study). All of them were evaluated by parasitological, clinical, and immunological criteria at the moment of diagnosis, at one month, and at three months after diagnosis. Due to ethical criteria, the control group (CG) was evaluated only at the moment of diagnosis to have references values. Twenty-two were men (46.8%) and 25 women (53.1%). Age analysis showed that 95% of the individuals were between 2 and 40 years old. Selection according to the clinical variant showed that 11 (23%) individuals corresponded to the cohort of AIA, 13 (28%) to the cohort of CIA, 13 (28%) to the cohort of AC, and 10 (21%) to the CG.

The clinical characterization at the moment of diagnosis from these cohorts showed: diarrhea with mucus and blood present in 5 cases of AIA; feces with mucus in 6 cases of AIA and 3 cases of CIA; colic in 9 cases of AIA and 10 cases of CIA; abdominal distension in 8 cases of AIA and 2 cases of CIA; tenesmus in 11 cases of AIA and 5 cases of CIA; constipation in 11 cases of CIA. After diagnosis, all the patients were treated and in each case the physician administered specific treatment according to the hospital regulations.

Identification of *E. histolytica/E. dispar* by colorimetric PCR. Control Group samples (10 out of 47) were negative by coproparasitoscopic analysis (CPS), and also by PCR. From the remaining 37 positive samples by CPS, only 29 were positive by colorimetric PCR for *E. histolytica* and/or *E. dispar* (Table 1). Based on PCR results, the clinically defined cohorts were further divided into subsets according to the presence or absence of *E. histolytica/E. dispar* (Table 2).

Immunological parameters. Antibody levels, BTI, CD4+/CD8+ ratio, IL-4, and INF-γ levels for the clinically defined cohorts, and the PCR defined subsets were determined at diagnosis time, and at one and three months after diagnosis.

Distribution of anti-Entamoeba spp. *antibodies.* A signifi-

TABLE 1
Determination of *Entamoeba histolytica/E. dispar* by colorimetric PCR in individuals with clinical variants of amoebiasis

Clinical variant	*PCR + E.h.	**PCR + E.d.	Negative	Total
Acute intestinal amoebiasis	7	2	2	11
Chronic intestinal amoebiasis	0	9	4	13
Asymptomatic carriers	2	9	2	13
Control group	0	0	10	10
Total	9	20	18	47

* = Identification of *Entamoeba histolytica* by PCR.
** = Identifcation of *Entamoeba dispar* by PCR.

cant difference was observed between AIA and CG at the time of diagnosis and at one month ($P < 0.05$), but not three months later (Figure 1). In the cohort of AIA, the *E. histolytica* subset was seropositive at the time of diagnosis; this titer decreased at one month but three months later it was even lower than that of CG (Table 2). With respect to the *E. dispar* subset, it presented low titers at the time of diagnosis with respect to CG (Table 2) but it increased at one month after diagnosis (0.137 ± 0.114).

Blast Transformation Index (BTI). The results obtained with the groups (AIA, CIA, AC) or the subsets (PCR positive for *E. histolytica* or *E. dispar*) did not show significant differences compared with CG (data not shown).

CD4+/CD8+ ratio. Determination of the CD4+/CD8+ ratio at the different times did not show significant differences among the different groups and subsets, compared with CG (data not shown).

Interleukin-4 and Interferon-γ (Th1/Th2 profile). Th1/Th2 profile was defined by INF-γ and IL-4 serum levels. IL-4 levels at the time of diagnosis and at three months were significantly different between CG and AIA (Figure 2), and also in the subset proven to be infected with *E. histolytica* ($P < 0.05$) (Table 2). The two members of the AIA subset infected with *E. dispar* did not show significantly raised levels of IL-4 (Table 2). Levels of INF-γ in two individuals from the AC cohort infected with *E. histolytica*, were significantly higher, both at diagnosis time and at three months compared with CG ($P < 0.05$) (Figure 3); the nine AC patients infected with *E. dispar* were not significantly different from CG (Table 2). Neither AIA cohort (Figure 3) nor those individuals from the subset infected with *E. histolytica* (Table 2), showed significant differences in serum INF-γ levels compared with CG.

DISCUSSION

The recognition of *E. histolytica* and *E. dispar* as different species, has provided important insights into the epidemic behavior of amoebiasis in the world,[1] and has had important epidemiological and clinical implications.[10] The existence of asymptomatic carriers of *E. histolytica,*[34–38] has raised doubts about the proportion of individuals infected who are truly at risk of invasive amoebiasis, and questions the human host factors, that favors this apparently commensal-like relationship with *E. histolytica*.

A mixture of symptomatic and asymptomatic cases is frequently observed in infections by pathogenic agents. Although some elements of the microorganisms such as virulence, inoculum size, exhibition routes, etc., affect the subsequent manifestations of the infection, undoubtedly host factors contribute in a substantial way in the outcome of the host-parasite relationship.[39] Among these factors, the CD4+/CD8+ ratio and a specific cytokine profile, characteristic of subpopulations of CD4+ T lymphocytes, seem to play an important role.[21,40] In the present study, we examined the importance of humoral and cellular immunity in the outcome of the relationship between the host and *E. histolytica/E. dispar* in individuals with clinical variants of amoebiasis.

The population studied was young (80% younger than 20 years old), and came from urban and suburban areas of the municipality of Puebla, México, an entity of 2 million inhabitants. The seroprevalence of 14.98% reported for the state of Puebla is relatively high compared with other parts of México[8] but comparable to those reported for other cities in developing countries such as South-Africa, Brazil, India, and Bangladesh.[41–43]

Infection with *E. histolytica/E. dispar* in endemic areas frequently coexists with other protozoa and helminths[35]; therefore, to avoid the interpretation biases observed in previous investigations[41] we included in the study only those cases infected with *E. histolytica/E. dispar*. By following them during three months, beginning at the moment of diagnosis, we were able to evaluate the behavior of different forms of the disease over time. The cohort of AIA comprised the cases of apparent amoebic dysentery referred from a suburban clinic over a 2-year period. The parasitological diagnosis consisted on the finding of trophozoites associated with the presence of mucus and /or blood, a widely used method effective in the diagnosis of AIA,[44] in accordance with recommendations of the World Health Organization (1997).[2] Parasites were later confirmed as *E. histolytica* by colorimetric PCR standardized using spiked samples.[17,30] This procedure is apparently 100% specific but not 100% sensitive when applied to stored fecal samples (Shire and Ackers, unpublished observations). Thus, whereas a positive result confirms the diagnosis, a negative one does not exclude it; therefore, an adequate sample storage and conservation procedure is of great importance. Based on PCR results, the clinically defined cohorts were further subdivided.

It should be noted that even before applying PCR, in 90% of the cases the doctor's clinical suspicion of amoebic dysentery was not corroborated, demonstrating a clinical overdiagnosis of AIA. It has been reported that, patients diagnosed with amoebic dysentery are frequently infected with *Shigella dysenteriae* and *Shigella flexneri*,[45] or other different pathogens including enteroinvasive *Escherichia coli*, *Salmonella* spp., and *Campylobacter jejuni.*[46]

The production of antibodies is the main immunological manifestation of invasive amoebiasis in humans, although its presence is not associated with resistance to infection or with protective immunity.[18] Their utility is accepted in cases of extra intestinal invasive amoebiasis, but not in the intestinal form.[18,47] In contrast with reports from other groups, in this study the cohort of AIA showed significantly higher antibody titers compared with those in the cohorts of CIA, AC, and CG, both at the moment of diagnosis and one month later; values had returned to control levels by three months. The humoral immune response observed with the different groups was obtained using total extract of *E. histolytica* trophozoites as the antigen. The possibility to use *E. dispar* instead of *E. histolytica* as the antigen, for those cases where PCR was positive for *E. dispar*, may change the results obtained so far.

TABLE 2

Subsets according to PCR diagnosis of *Entamoeba histolytica/E. dispar* and its correlation with immunological parameters at the moment and three months after diagnosis

	Clinical variant					
	AIA		CIA	AC		CG
	Eh	Ed	Ed	Ed	Eh	Neg
PCR	7	2	9	9	2	10
	At moment of diagnosis					
ELISA	**0.149 ± 0.053***	0.069 ± 0.038	0.093 ± 0.057	0.069 ± 0.033	0.125 ± 0.033	0.073 ± 0.039
IL-4	**31.56 ± 3.28***	27.76 ± 1.54	29.07 ± 3.49	29.53 ± 3.68	30.76 ± 4.05	27.72 ± 2.81
INF-γ	11.56 ± 3.95	8.04 ± 0.22	11.13 ± 5.85	11.40 ± 3.22	**69.24 ± 16.32***	9.59 ± 1.84
	At 3 months after diagnosis					
ELISA	0.067 ± 0.041	0.071 ± 0.018	0.092 ± 0.043	0.082 ± 0.056	0.161 ± 0.091	0.073 ± 0.039
IL-4	**31.91 ± 4.31***	28.72 ± 2.13	27.98 ± 5.44	30.53 ± 3.42	25.73 ± 2.22	27.72 ± 2.81
INF-γ	11.41 ± 8.36	9.58 ± 0.22	9.18 ± 0.97	9.89 ± 2.53	**39.44 ± 7.35***	9.59 ± 1.84

AIA: Acute Intestinal Amoebiasis; CIA: Chronic Intestinal Amoebiasis; AC: Asymptomatic Carrier, CG: Control Group. PCR: shows the distribution by subsets of positive cases for *E. histolytica* (Eh) and *E. dispar* (Ed) according to PCR results. ELISA: Determination of anti-*E. histolytica* antibodies expressed as O.D. Reference values: 0.032 ± 0.009 O.D. IL-4: Seric levels expressed in pg/mL. Reference values: 27.59 ± 2.81 pg/mL. INF-γ: Seric levels expressed in pg/mL. Reference values: 9.45 pg/mL. *, $P < 0.05$ (Mann-Whitney U test, compared with CG).

Interestingly, the two patients assigned to the AIA cohort on clinical grounds but in whom PCR demonstrated *E. dispar* presented lower antibody levels compared with patients with AIA and *E. histolytica*.

Regarding cell-mediated immunity the work of Savant,[34] Ortiz,[48] and later Salata[49] observed a poor lymphocyte response measured by BTI in circulating T lymphocytes. On the other hand, Ganguly and others,[50] demonstrated a decrease in the number of T lymphocytes circulating in the blood of patients with amoebic hepatic abscess. In our investigation, the proliferation of T cells measured by BTI was low. This differs from the results of Ortiz, Harris, Segovia, and Diamanstein[51–54] who found a proliferative response to amoebic antigens from lymphocytes of patients with amoebic hepatic abscess. Moreover, Velásquez and others,[55] observed proliferation of peripheral blood mononuclear cells of a patient with amoebic hepatic abscess against a 170 kDa recombinant protein. In our case we used a total extract of *E. histolytica*, and Talamás-Rohana and others,[28] have reported that whole extract might contain growth inhibiting factors, explaining in part the results obtained by other authors,[34,48,49] as well as the low levels of blast transformation found in all the clinical variants tested in this study, including the CG.

Salata and others[49] have showed a decrease of CD4+ helper T cells and an increase of CD8+ cells in patients with amoebic hepatic abscess. In our study the patients with invasive amoebiasis showed values for the CD4+/CD8+ ratio that were lower than those reported previously. Suppression of the cellular immune response has been observed during the infection by protozoan parasites, including *Leishmania donovani*[56] and *Trypanosoma cruzi*.[23] There is also evidence that the response of T cells is systematically suppressed during the acute illness due to *E. histolytica*, as has been observed in animal models[57] and in patients with amoebic hepatic abscess.[49] Serum from *E histolytica*-infected gerbils[58] and immune sera from patients[25] selectively suppress T cell proliferation by inhibiting IL-2 and IFN-γ production. Previous studies have shown that in infections caused by pathogens that induce immunosuppression in the host, such as *L. donovani*,[56] HIV,[59] and *T. cruzi*,[23] the outcome of the relationship could be regulated by CD4+ helper T cells with different pattern of cytokines. Protective immunity would be associated with Th1 cells that produce INF-γ and IL-2, whereas Th2 cells, which produce immunosuppressive IL-4 and IL-10, could facilitate the survival and dissemination of the parasite generating tissue damage and the appearance of the disease.[60]

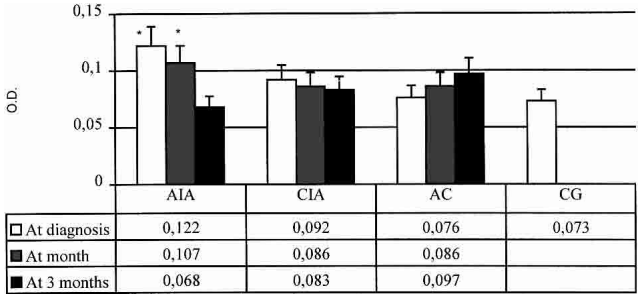


FIGURE 1. Anti-*E. histolytica* antibodies levels. Sera from each group were evaluated by ELISA using the same serum dilution (1:100). AIA: Acute Intestinal Amoebiasis; CIA: Chronic Intestinal Amebiasis; AC: Asymptomatic Carrier; CG: Control Group. Serology: Determination of anti-*E. histolytica* antibodies expressed as O.D. Reference value: 0.032 ± 0.009 O.D. *, $P < 0.05$ (Mann-Whitney *U* test, compared with CG).

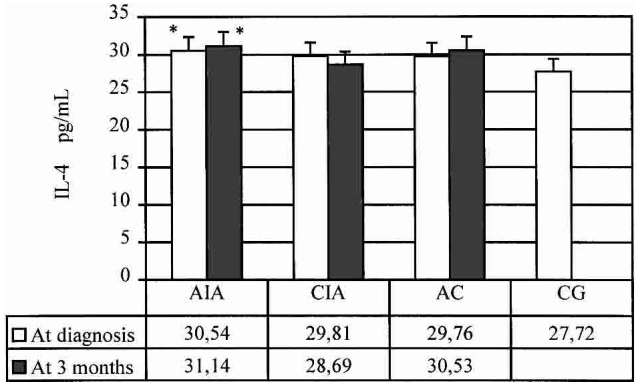
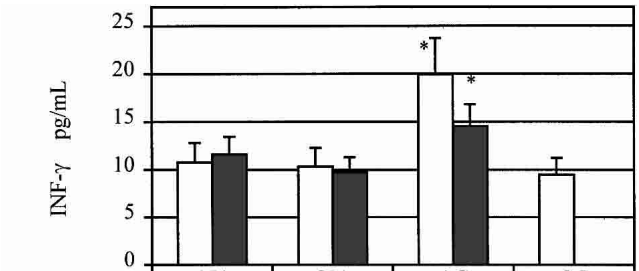


FIGURE 2. Determination of seric IL-4 levels. Sera from each group were evaluated by ELISA. AIA: Acute Intestinal Amoebiasis; CIA: Chronic Intestinal Amoebiasis; AC: Asymptomatic Carrier; CG: Control Group. IL-4: Seric levels expressed in pg/mL. Reference value: 27.59 ± 2.81 pg/mL. *, $P < 0.05$ (Mann-Whitney *U* test, compared with CG).



	AIA	CIA	AC	CG
At diagnosis	10,77	10,31	19,94	9,45
At 3 months	11,59	9,73	14,5	

FIGURE 3. Determination of seric INF-γ levels. Sera from each group were evaluated by ELISA. AIA: Acute Intestinal Amoebiasis; CIA: Chronic Intestinal Amoebiasis; AC: Asymptomatic Carrier; CG: Control Group. INF-γ: Seric levels expressed in pg/mL. Reference value: 9.45 pg/mL. *, $P < 0.05$ (Mann-Whitney U test, compared with CG).

Recent evidence[61–63] suggests an important role for IL-10, but not for IL-4 in the Th2 response. However, as has been proposed by others,[64–67] we analyzed IL-4 and IFN-γ as indicators of Th2 and Th1, respectively. These subpopulations may in part explain the behavior observed in the cohorts of patients with invasive amoebiasis and asymptomatic carriers, considered in this study. The first group presented significantly high levels of IL-4 (Th2 profile) at the moment of the diagnosis, associated with the presence of the pathogenic species *E. histolytica* (determined by PCR), and returning to normal levels, compared with CG, at three months post-therapy. Regarding the cohort of untreated asymptomatic carriers, the two individuals infected with *E. histolytica* presented high levels of INF-γ at the moment of diagnosis, maintaining these levels for the following three months, without alteration in the other evaluated parameters, (levels of antibodies, BTI, CD4+/CD8+ ratio). The nine AC patients infected with *E. dispar* were not different from the CG in any immunological parameters and it is tempting (although the numbers are very small) to attribute asymptomatic carriage of pathogenic *E. histolytica* to a powerful Th1 response not seen in those who succumbed to amoebic dysentery.

It is recognized that the chronic abdominal pain and intestinal alterations are symptoms experienced by 15% of apparently healthy people.[68] In endemic areas such as México, this type of condition is frequently diagnosed as chronic intestinal amoebiasis. The etiology of CIA has not been settled and clear-cut differences between this disease and the syndrome of irritable bowel have not been defined.[69,70] Infection in all the cases where it could be determined in the cohort of CIA, (characterized by abdominal distension, colicky pain, tenesmus, alternating diarrhea and constipation, and positive serology) was due to *E. dispar*. Studies carried out in India,[71] Panama,[72] Puerto Rico,[73] and Honduras[74] have shown that the typical abdominal symptoms of CIA were observed more frequently in individuals negative for amoeba in feces.[75] On the other hand, individuals with diagnosis of CIA treated with anti-amoebic drugs showed no improvement of symptoms.[76]

In this study, the identification of the non-pathogenic *E. dispar* in all the cases of patients classified as CIA, does not provide evidence for a causal relationship between infection and symptoms. This suggests reconsideration of the existence of this type of clinical variant of amoebiasis.

In general, our results show that invasive amoebiasis, induced by the presence of *E. histolytica* could be partly explained by the levels of the IL-4 associated with a Th2 cytokine profile. Resistance to the invasive form of the illness in asymptomatic carriers of *E. histolytica* may be explained by the high levels of IFN-γ associated with a Th1 cytokine profile, as found in this investigation.

The identification of *E. histolytica* in asymptomatic carriers demonstrates the importance of routinely making a species-specific diagnosis in view of the participation of the asymptomatic carriers of *E. histolytica* in the establishment and maintenance of a pathogenic cycle of extra-intestinal amoebiasis in endemic regions.

To have elements to better understand the clinical-epidemiological pattern of amoebiasis, it will be necessary to perform longitudinal studies with a larger number of patients to analyze all the immunological parameters which participate in the pathogenic process associated with *E. histolytica* infection.

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