http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (20): 1376-1380, 2009
ISSN 1028-8880

Th-1/Th-2 Cytokine Pattern in Human Amoebic Colitis

[1]A. Rafiei, [2]A. Ajami, [3]M. Hajilooi and [4]A. Etemadi
[1]Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[2]Department of Immunology and Microbiology, Sari Medical School,
Mazandaran University of Medical Sciences, Sari, Iran
[3]Department of Pathobiology, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
[4]Department of Infectious diseases, Faculty of Medicine,
Jondy-Shapour University of Medical Sciences, Ahwaz, Iran

Abstract: Amoebiasis, caused by *Entamoeba histolytica,* is still considered a major health problem in developing countries. Since the immune response during human amoebiasis has not been clearly defined, we chose to evaluate cytokine production in patients suffering from amoebic colitis. A case-control association study was carried out on 62 subjects, including 31 patients with amoebic colitis and 31 healthy controls (age, sex and geographic region-matched). Serum levels of IL-12, IFN-γ, IL-13 and IL-5 were measured by solid-phase sandwich enzyme linked immunosorbant assay. Serum levels of IFN-γ, IL-12, IL-13 and IL-5 were higher in the patients with amoebic colitis than in healthy controls, but were only statistically increased for IL-5 ($p = 0.04$) and IL-13 ($p = 0.014$). Stratification of patients according to gender revealed that IL-13 was significantly elevated in men as compared to levels measured in women ($p = 0.04$). These findings suggest that *E. histolytica* induce a mixed Th-1/Th-2 response with a polarization toward Th-2 during the early stage of amoebiasis, which may aide in developing a clinical illness.

Key words: Amoebic colitis, IL-5, IL-12, IL-13, IFN-γ

INTRODUCTION

Entamoeba histolytica is an enteric dwelling human protozoan parasite that causes the disease amoebiasis, which is endemic in the developing world. Estimates suggest that 40-50 million cases of amebic colitis and liver abscess occur annually in the world, which result in 40,000-110,000 deaths (WHO/PAHO/UNESCO, 1997). Intestinal amoebiasis is the most common disease caused by *E. histolytica.* It is characterized by invasion of the intestinal mucosa, which causes ulcerative colitis.

Both humoral and Cell-Mediated Immunity (CMI) have been implicated in the pathogenesis of amoebiasis, the latter playing the dominant role (Trissl, 1982; Guo *et al*., 2009). It has been shown that cytokines orchestrate the immune response and influence the outcome of the disease. Therefore, much attention is now focused on the role of cytokines, which modify the immune response to various infections (Denis and Chadee, 1989; Tallima *et al*., 2009). Studies examining the immune response during human amoebiasis are lacking (González-Amaro *et al*., 1994).

Despite the importance of amoebiasis in terms of public health burden, the lack of integrated data on immunopathogenesis means that we have only an incomplete description of the initiation and development of amoebic colitis. To date, most studies determining host resistance mechanisms to *E. histolytica* have been done using a hamster model of disease. Hamsters with an amoeba infection mount a vigorous Th-1 response, driven by high levels of IL-12, IL-2 and IFN-γ. However, a Th-2 type immune response deteriorates the disease and leads to chronic disease. Previous studies have indicated that CMI mediated by IFN-γ plays a major role in immunity against amoebiasis (Guo *et al*., 2009) and may predict future susceptibility to symptomatic amoebiasis (Haque *et al*., 2007). During invasive amoebiasis, studies of cellular immune responses have shown decreases of CD^{4+} cells, increases of CD^{8+} cells and decreases in the *in vitro* proliferation of T lymphocytes against amoebic antigen, indicating a suppression of the immune response (Salata *et al*., 1990). Previous research has shown that antigenic epitopes of *E. histolytica* can stimulate either the Th-1 or Th-2 immune responses (Mann *et al*., 1993). In

Corresponding Author: A. Rafiei, Department of Immunology and Microbiology, Faculty of Medicine,
Mazandaran University of Medical Sciences, 18 KM Khazar Blvd, Khazar Sq. Sari, Iran
Tel: +98-151-3543088 Fax: +98-151-3543087

humans, the mechanisms are less well-defined and it appears that a balance between the Th-1 and Th-2 responses might present the optimal response.

The present study was conducted to evaluate IL-12, IFN-γ, IL-5 and IL-13 production in individuals suffering from amoebic colitis to demonstrate the role of Th-1 and Th-2 responses in immunity during human amoebiasis.

MATERIALS AND METHODS

The study consisted of 31 (10 females and 21 males, mean age 23.7±15.2 years) patients with clinical and laboratory diagnosis of amoebic colitis from areas of Northern Iran, who referred to the Razi Hospital of Mazandaran University of Medical Sciences during September 2007 and June 2008. Detailed medical records, including demographic data, disease status, clinical presentation and laboratory findings were reviewed. Control subjects included 35 clinically healthy individuals residing in the same geographic area and were age- and sex-matched subjects without inflammatory or autoimmune diseases. Controls were also CRP and stool exam negative and did not receive any medication. Serum from each individual was obtained by centrifugation at 1600 g for 15 min. Serum samples were stored at -70°C until analyzed. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by Mazandaran University of Medical Sciences medical research committee. Informed consent was obtained from all patients and controls enrolled in the study.

Parasitological examination: Three consecutive stool samples were withdrawn from each individual and examined for the presence of *Entamoeba* by saline preparation and iodine staining. All negative stool samples were subjected to the zinc sulphate floatation concentration technique (Campbell and Chadee, 1997) and examined for *Entamoeba* trophozoite and cysts. Patients were considered to have amoebic colitis if trophozoites were found in stool samples (Kretschmer and López-Osuna, 1990). Since, *E. histolytica* and *E. dispar* cannot be differentiated microscopically, all microscopy positive samples were evaluated by a TechLab *E. histolytica* stool antigen detection ELISA test (TechLab, Inc., Blacksburg, VA).

Cytokine assays: Cytokines were detected in the serum by using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits with paired cytokine-specific monoclonal antibodies according to the manufacturer's recommended procedure (Bender Med Systems; Vienna, Austria). The detection limit for IL-5, IL-10, IL-12 and IFN-γ were 1.46, 0.99, 2.1 and 0.99 pg mL^{-1}, respectively.

Statistical analysis: Statistical analyses were performed using SPSS 10.0 for Windows (SPSS, Chicago, Illinois). Statistical significance of continuous variables was determined by using a Student's T test. Continuous variables were reported as Mean±standard deviation (SD). All tests were two-tailed and $p<0.05$ was considered statistically significant.

RESULTS

Study population: The χ^2 test demonstrated that the number of males and females were not significantly different in the patients and controls (21 versus 10 and 16 versus 15, p = 0.3, respectively). The ages of the two groups were also not significantly different (23.7±15.2 and 28.35±11.97, p = 019). No significant differences in white blood cells and platelet counts or hemoglobin concentration between the groups were observed (Table 1). The most common symptoms among amoebisis patients were abdominal pain, dysentery and abdominal

Table 1: Characteristics of study population

	Patients	Controls	p-value
Gender (M/F)	21/10	16/15	0.3
Age (Mean±SD)	23.7±15.2	28.35±11.97	0.19
White blood cell (mL^{-1})	$7.55\times10^3\pm1.84\times10^3$	$5.12\times10^3\pm1.94\times10^3$	0.4
Platelet (μL^{-1})	$230\times10^3\pm32.27\times10^3$	$223\times10^3\pm50.78\times10^3$	0.8
Hemoglobin (g dL^{-1})	12.9±1.87	13.5±1.72	0.09
Clinical manifestations (%)			
Fever (>38°C)	36	5	<0.0001
Anorexia	38.5	2	<0.0001
Abdominal pain	92	ND.	
Headache	30.8	7	<0.0001
Dysentery	80	ND.	
Mucobloody diarrhea	30.8	ND.	
Abdominal distention	69.2	2	<0.0001
Weight loss	40	1	<0.0001
Stool findings (%)			
Trophozoites	98	ND.	
Hematophagic trophozoites	75	ND.	
Cysts	20	2	<0.0004

ND: Non detectable

Table 2: Cytokine levels in serum of patients with amoebic colitis and healthy controls

	IL-12	IL-13	IFN-γ	IL-5
	(pg mL^{-1})			
Amoebiasis	12.96±8.6	11.070±7.86	6.10±5.46	9.20±3.24
Controls	7.20±5.45	2.570±1.61	3.77±3 38	3.17±1.08
p-value	0.27	0.014	0.33	0.04

Table 3: Gender differences in cytokine levels in serum of patients with amoebiasis

	IL-12	IL-13	IFN-γ	IL-5
	(pg mL^{-1})			
Male	20.32±4.19	20.18±7.35	3.33±1.77	7.24±1.95
Female	12.52±5.19	6.60±2.78	6.47±3.02	14.11±4.2
p-value	0.28	0.04	0.48	0.10

distention. None of the patients had peritonitis, empyema, pericarditis, chronic colitis and liver or brain abscess complications.

Serum levels of IL-5, IL-12, IL-13 and IFN-γ in amoebiasis patients and controls: To determine the role of Th-1/Th-2 responses in human amoebiasis, we studied serum concentrations of IL-5 and IL-13 (Th-2-type), IFN-γ (Th-1-type) and also IL-12 in patients with amoebic colitis and healthy controls. As shown in Table 2, the serum levels of all cytokines tested were higher in patients than in controls, but only IL-5 and IL-13 levels were statistically significant.

It was previously reported that prevalence of amoebiasis is far more common in males than females (Denis and Chadee, 1989). However, further analysis was conducted comparing cytokine levels in male and female patients. As shown in Table 3, only serum levels of IL-13 were significantly higher in infected males than in females.

DISCUSSION

In the present study, the serum levels of IL-5 and IL-13 were significantly higher in patients with amoebic colitis as compared to healthy controls. Resistance to amoebiasis requires an effective cell-mediated immune response against *E. histolytica*, which is mediated by nitric oxide released from activated macrophages. The pathogenicity of *E. histolytica* results from its adhesion to the intestinal epithelium followed by its penetration into the mucosa (Kretschmer and López-Osuna, 1990). During mucosal invasion, the activation of the immune response is induced via the release of inflammatory cytokines by host epithelial cells that initiate an acute inflammatory process (Seydel *et al*., 1997). However, inflammation induced by amoeba infection in the intestine, is driven by a Th-2 cell phenotype. Polarization of the immune response toward Th-2 type, which presents by production of IL-5, IL-13 and IL-4 (Chatelain *et al*., 1999), has various effects on amoeba pathogenesis. First, production of these cytokines can down regulate macrophage parasitocidal activity, which may result in tissue damage by the release of necrotizing enzymes (Virk *et al*., 1990). This phenomenon helps to an invasive effort in predisposed individuals (Murray, 1998). It has been reported that parasite secreted products are likely to regulate macrophage mediated responses to *E. histolytica* trophozoites (Salata *et al*., 1989). Therefore, *E. histolytica* suppresses both the macrophage respiratory burst and antigen presentation by class II Major-Histocompatibility-Complex (MHC) molecules (Haque *et al*., 2003). Second, the induction of mucosal inflammation driven by Th-2 cytokines helps to contain the amoebic infection at an early stage, at the cost of extensive mucosal destruction. Third, high production of IL-5 in combination with IL-10 promotes IgA class switching and maturation in the late stage of amoebic colitis and thus, induction of protective mucosal immunity (Corthesy, 2007). It has been suggested that intestinal antilectin IgA antibodies could prevent parasite colonization of the gut (Ravdin *et al*., 2003). It has also been demonstrated that depletion of CD^{4+} T lymphocytes in susceptible mice results in a reduction of mastocyte infiltration to the submucosa and a diminished secretion of IL-4 and IL-13 (Houpt *et al*., 2002). Consequently, a reduction in parasite burden was also observed. On the contrary, occurrence of an acute inflammatory reaction in the early stage of intestinal amoebiasis, which orchestrates with Th-2 cytokines, is a double edged sword.

The Th1/Th2 polarization paradigm, yet biologically questionable in humans (Rook, 2001) follows many complex and changing responses to environmental factors and, on the other hand, depends on the genetic background of the cells. Th-1 driven responses appear to be responsible for the outcome and the protective immunity in resolved invasive amoebiasis (Kretschmer and López-Osuna, 1990). Indeed, activation of CD4+ T cells by macrophages is pivotal for an effective specific immune response, requiring mainly a Th-1 type cytokine secretion, such as IFN-γ and IL-12 [5, 6]. Decreases in IFN-γ and IL-12 in patients with amoebiasis could represent suppression of the protective Th-1 responses in acute amoeba colitis. Otherwise, amoebic components suppress NO production by macrophages during a primary infection (Seguin *et al*., 1997) and cytokine induction in the late phase of amebic abscess of the liver (Bekker-Mendez *et al*., 2006). Previous research determined that failure of macrophage activation due to suppression of Th-1 responses causes rapid spread of *E. histolytica*, as well as increased multiplication (Campbell and Chadee, 1997). This interpretation was

confirmed by detecting increased IFN-γ in the patients with amoeba liver abscess after treatment (Sanchez-Guillen *et al*., 2002).

Interleukin-13 has been shown to have potent anti-inflammatory properties and can down-regulate macrophage functions such as production of IL-12, expression of inducible nitric oxide synthase, as well as up-regulation of IL-10 production (Wills-Karp *et al*., 1998). Significant increases in IL-13 production in amoeba patients may explain the low IL-12 production. Indeed, high production of IL-5 and IL-13 in patients with intestinal amoebiasis is in agreement with a previous study that reported increases in IL-4 and IL-10 production in symptomatic patients (Bansal *et al*., 2005). Paradoxically, IL-13 and also IL-4 may have the potential to systemically induce immune suppression in *E. histolytica* infected patients and thereby aide in developing a clinical illness. These findings are in agreement with the results of an *in vitro* study that demonstrated that *E. histolytica* induces increases in IL-1β, IL-2, IL-5, IL-6 and IL-10 production in axenic culture (Salata *et al*., 1989). In addition, high IL-13 levels in male patients compared to females may be partially responsible for the high prevalence of disease in these patients (Acuna-Soto *et al*., 2000).

ACKNOWLEDGMENTS

We are grateful to Dr Alian, Department of Infectious Diseases, Razi Hospital, Ghaemshar, Iran; for their help in providing samples.

REFERENCES

Acuna-Soto, R., J.H. Maguire and D.F. Wirth, 2000. Gender distribution in asymptomatic and invasive amebiasis. Am. J. Gastroenterol., 95: 1277-1283.

Bansal, D., R. Sehgal, Y. Chawla, N. Malla and R.C. Mahajan, 2005. Cytokine mRNA expressions in symptomatic vs. asymptomatic amoebiasis patients. Parasite Immunol., 27: 37-43.

Bekker-Mendez V.C., V.L. Pérez-Castillo, M.G. Rico-Rosillo, M. Pérez-Rodríguez and J. Arellano-Blanco *et al*., 2006. Downregulation of selected cytokines in amebiasis. Arch. Med. Res., 37: 556-558.

Campbell, D. and K. Chadee, 1997. Interleukin (IL)-2, IL4 and tumour necrosis factor alpha augments nitric oxide dependent macrophages cytotoxicity against *Entamoeba histolytica* liver abscess development in gerbils. J. Infect. Dis., 175: 1176-1183.

Chatelain, R., S. Mauze and R.L. Coffman, 1999. Experimental Leishmania major infection in mice: Role of the IL-10. Parasite Immunol., 21: 211-218.

Corthesy, B., 2007. Roundtrip ticket for secretory IgA: Role in mucosal homeostasis. J. Immunol., 178: 27-32.

Denis, M. and K. Chadee, 1989. Cytokine activation of murine macrophages for *in vitro* killing of *Entamoeba histolytica* trophozoite. Infect. Immun., 57: 1750-1756.

González-Amaro, R., S. Andrade, L. Baranda, C. Abud-Mendoza, D.P. Portales, B. Moncada and L. Llorente, 1994. Plasma levels and *in vitro* production of tumor necrosis factor-alpha and interleukin-6 in patients with amebic. Rev. Invest. Clin., 46: 209-213.

Guo, X., L. Barroso, S.M. Becker, D.M. Lyerly and T.S. Vedvick *et al*., 2009. Protection against intestinal amebiasis by a recombinant vaccine is transferable by T cells and mediated by gamma interferon. Infect Immun., 77: 3909-3918.

Haque, R., C.D. Huston, M. Hughes, E. Houpt and W.A.J.R. Petri, 2003. Amoebiasis. N. Engl. J. Med., 348: 1565-1573.

Haque, R., D. Mondal, J. Shu, S. Roy and M. Kabir *et al*., 2007. Correlation of interferon-gamma production by peripheral blood mononuclear cells with childhood malnutrition and susceptibility to amebiasis. Am. J. Trop. Med. Hyg., 76: 340-344.

Houpt, E.R., D.J. Glembocki, T.G. Obrig, C.A. Moskaluk and L.A. Lockhart *et al*., 2002. The mouse model of amebic colitis reveals mouse strain susceptibility to infection and exacerbation of disease by CD4+ T cells. J. Immunol., 169: 4496-4503.

Kretschmer, R.R. and M. López-Osuna, 1990. Effector Mechanisms and Immunity to Amebas. In: Amebiasis: Infection and Disease, Histolytica, E. and R. Kretschmer (Eds.). CRC Press, Boca Raton, pp: 105-122.

Mann, B.J., C.N. Chung, J.M. Dodson, L.S. Ashley, L.L. Braga and T.L. Snodgrass, 1993. Neutralizing monoclonal antibody epitopes of the *Entamoeba histolytica* galactose adhesin map to the cysteine-rich extracellular domain of the 170-kilodalton subunit. Infect. Immun., 61: 1772-1778.

Murray, J.S., 1998. How the MCM selects Th1/Th2 immunity. Immunol. Today, 19: 157-163.

Ravdin, J.I., M.D. Abd-Alla, S.L. Welles, S. Reddy and T.F.G.H. Jackson, 2003. Intestinal antilectin immunoglobulin A antibody response and immunity to *Entamoeba dispar* infection following cure of amebic liver abscess. Infect. Immun., 71: 6899-6905.

Rook, G., 2001. Th1 or Th2-cell commitment during infectious disease: An oversimplification. Trends Immunol., 22: 481-482.

Salata, R.A., P. Ahmed and J.I. Ravdin, 1989. Chemoattractant activity of *Entamoeba histolytica* for human polymorphonuclear neutrophils. J. Parasitol., 75: 644-648.

Salata, R.A., A. Martinez-Palomo, L. Canales, H.W. Murray, N. Trevino and J.I. Ravdin, 1990. Suppression of T lymphocytes responses to *Entamoeba histolytica* antigen by immune sera. Infect. Immun., 58: 3941-3946.

Sanchez-Guillen, M.C., R. Perez-Fuentes, H. Salgado-Rosa, A. Ruiz-Argüelles, A. Ackers, A. Shire and P. Talamas-Rohana, 2002. Differentiation of *Entamoeba histolytica* and *Entamoeba dispar* by PCR and their correlation with humoral and cellular immunity in individuals with clinical variants of amoebiasis. Am. J. Trop. Med. Hyg., 66: 731-737.

Seguin, R., B.J. Mann, K. Keller and K. Chadee, 1997. The tumor necrosis factor alpha-stimulating region of galactose-inhibitable lectin of *Entamoeba histolytica* activates gamma interferon-primed macrophages for amebicidal activity mediated by nitric oxide. Infect. Immun., 65: 2522-2527.

Seydel, K.B., E. Li, P.E. Swanson and S.L.J.R. Stanley, 1997. Human intestinal epithelial cells produce proinflammatory cytokines in response to infection in a SCID mouse-human intestinal xenograft model of amebiasis. Infect. Immun., 65: 1631-1639.

Tallima, H., M. Salah, F.R. Guirguis and R. El-Ridi, 2009. Transforming growth factor-beta and Th17 responses in resistance to primary murine schistosomiasis mansoni. Cytokine,

Trissl, D., 1982. Immunology of *E. histolytica* in human and animal hosts. Rev. Infect. Dis., 4: 1154-1184.

Virk, K.J., N.K. Ganguly, R.N. Prasad, J.B. Dilawari and R.C. Mahajan, 1990. Kupffer cell functions during intestinal amoebiasis in guinea pigs. J. Gastro. Hepatol., 5: 518-524.

WHO/PAHO/UNESCO, 1997. Report of a consultation of experts on amoebiasis. Weekly Epidemiol. Rep. World Health Org., 72: 97-99.

Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schofield, T.Y. Neben, C.L. Karp and D.D. Donaldson, 1998. Interleukin-13: Central mediator of allergic asthma. Science, 282: 2258-2261.