Parasitol Res (2012) 111:487–491
DOI 10.1007/s00436-012-2830-0

SHORT COMMUNICATION

# Findings related to IL-8 and IL-10 gene polymorphisms in a Mexican patient population with irritable bowel syndrome infected with *Blastocystis*

**Angelica Olivo-Diaz · Mirza Romero-Valdovinos · Areli Gudiño-Ramirez · Jesus Reyes-Gordillo · Diego Emiliano Jimenez-Gonzalez · Maria Elena Ramirez-Miranda · Williams Arony Martinez-Flores · Fernando Martinez-Hernandez · Ana Flisser · Pablo Maravilla**

Received: 24 November 2011 / Accepted: 16 January 2012 / Published online: 28 January 2012


**Abstract** The intestinal protozoan parasite *Blastocystis* is one of the most common parasites worldwide in humans and, although its ability to cause human disease has been questioned, some reports have demonstrated that this microorganism is associated to the development of irritable bowel syndrome (IBS) and to a proinflammatory response, in which the expression of some cytokines is unregulated. Since inflammatory cytokine gene polymorphisms might have a role in the pathophysiology of IBS, we assessed the role of single nucleotide polymorphisms (SNPs) for interleukin (IL)-8 and IL-10, in previously collected DNA samples from IBS patients and controls, with or without *Blastocystis* infection. IL-8+396(G) and IL-10-1082 (A) alleles ($p$=0.0437 and $p$=0.0267, respectively), as well as their homozygous ($p$<0.0001 and $p$=0.0039, respectively) and IL-8+781(CT) ($p$=0.0248) genotypes were significantly overrepresented in patients with IBS in comparison with controls. IL-8+396(GG) genotype was relevant because it was associated to IBS ($p$<0.0001), to *Blastocystis* ($p$=0.0025), and to IBS–*Blastocystis* ($p$=0.0272). In the latter binomial association, this genotype presented a high contribution (etiological fraction=0.452) and a risk >fourfold to develop IBS. IL-8+781 (T) and IL-10-592 (C) alleles were also associated to *Blastocystis* and to IBS–*Blastocystis*, respectively ($p$=0.0448 and $p$=0.0166). Our results suggest that some IL-8 and IL-10 SNPs could change individual susceptibility increasing the relative risk in the development of IBS in *Blastocystis* carriers.

A. Olivo-Diaz · M. Romero-Valdovinos · A. Gudiño-Ramirez · J. Reyes-Gordillo · D. E. Jimenez-Gonzalez · M. E. Ramirez-Miranda · W. A. Martinez-Flores · F. Martinez-Hernandez · P. Maravilla (✉)
Hospital General "Dr. Manuel Gea Gonzalez",
Direccion de Investigacion,
Mexico, DF 14080, Mexico
e-mail: maravillap@yahoo.com

A. Flisser
Departamento de Microbiologia y Parasitologia,
Facultad de Medicina, Universidad Nacional Autonoma de Mexico,
Mexico, DF 04536, Mexico

## Introduction

*Blastocystis* is a parasite associated to the development of cutaneous and intestinal disorders as irritable bowel syndrome (IBS) (Yakoob et al. 2004, 2010; Tan 2008; Stensvold et al. 2009a; Tan et al. 2010; Jimenez-Gonzalez et al. 2011). IBS is one of the most common gastrointestinal diagnoses seen by primary care providers and gastroenterologists, in which abdominal pain or discomfort is associated with a change in bowel habits and disordered defecation (Longstreth et al. 2006). Although the definition of IBS states that symptoms are not due to active inflammation, transient mucosal inflammation is considered to be an important factor for the manifestation of this syndrome (Hotoleanu et al. 2008). In addition, some experimental infections in rats and in vitro assays have shown that *Blastocystis* can elicit a proinflammatory response, upregulating the expression of interferon-γ, interleukin (IL)-12, and tumor necrosis factor-alpha (TNF-α) (Iguchi

et al. 2009), as well as an early production of IL-8 (Long et al. 2001; Puthia et al. 2008).

Studies performed in Dutch and Iranian IBS patients demonstrated that certain genetic polymorphisms related to some proinflammatory ILs were associated with susceptibility to this disease (van der Veek et al. 2005; Barkhordari et al. 2010a, b). In a recent study, our group found that *Blastocystis* carriers have twofold increased relative risks for IBS, while gene promoter single nucleotide polymorphisms (SNPs) of IL-6 and TNF-α genes had similar frequencies in patients and controls; thus, we decided to assess the role of IL-8 and IL-10 SNPs.

## Material and methods

### Subjects

Forty-five IBS patients with mean age of 46.5±14.3, diagnosed according to Rome III criteria and 45 unrelated controls with mean age of 52.2±14.4, referred to the gastroenterology area of the Hospital General "Dr. Manuel Gea Gonzalez" between 2008 and 2009 were studied. Exclusion criteria for both groups were the presence of intestinal viruses or bacteria that cause diarrhea, parasites other than *Blastocystis* such as *Entamoeba histolytica/dispar*, *Giardia*, *Endolimax*, and *Hymenolepis* and the presence of an organic cause of disease, evident psychological alterations, social problems, pregnancy, dependence on analgesics, ulcerative colitis, tumors, or diverticulitis. *Blastocystis* diagnosis by PCR (Stensvold et al. 2006) was performed in all participants and showed that 14 patients (31%) and 6 controls (13%) were positive. *Blastocystis* subtypes (ST) detected were ST1 and ST3 similarly distributed between the IBS and control groups; the presence of IBS did not correlate to any particular ST (Jimenez-Gonzalez et al. 2011). This study was approved by the ethical and research committee of our hospital and a written informed consent was obtained from each participant, who afterwards, underwent a full physical examination and provided biological samples.

### Genotyping

DNA was obtained from 10 mL EDTA-treated peripheral blood using proteinase K and phenol/chloroform extraction (Sambrook et al. 2001). Typing of the IL8 polymorphism was achieved by PCR with the primers IL8(−251)F (5′-TCTAACACCTGCCACTCTAG-3′), IL8(−251)R (5′-CCTGAGTCATCACACTTCCT-3′), IL8(+396)F (5′-CTCTGTGTGAAGGTAAGCAC-3′), IL8(+396)R (5′-TATCAACAGGCACAGCTCTG-3′), IL8(+1633)F (5′-GGAAGAGAGCTCTGTCTGGA-3′), and IL8(+1633)R (5′-GATCCTGGCTAGCAGACTAG-3′) that generate fragments of 211, 798, and 988 bp, respectively. For IL8 (−251) polymorphism, the probes IL8(−251)A (5′-CATACATTTGATAATTCA-3′) and IL8(−251)T (5′-CATACAATTGATAATTCA-3′) were used. The primer IL8 (+396) amplified two polymorphisms that may be defined with the probes IL8(+396)G (5′-ATGCATGCTACATGGTATAA-3′), IL8(+396)T (5′-ATGCATGCTAAATGGTATAA-3′), IL8(+781)C (5′-ACATTGAACGACTTCCTAT-3′), and IL8(+781)T (5′-ACATTGAACAACTTCCTAT-3′). Alleles at position 1633 were defined with the probes IL8 (+1633)T (5′-GACATGAGTACAACAAACT-3′) and IL8 (+1633)C (5′-GACATGGGTACAACAAACT-3′). For the IL-10 polymorphisms, primers and probes for SNPs at positions −592, −819, and −1082 described by Meenagh et al. (2002) were used. IL-8 and IL-10 probes were labeled with digoxigenin-11-ddUTP, and alleles were identified by dot blot analysis with chemiluminescence as in Fajardo-Dolci et al. (2006).

### Statistical analysis

Allele and genotype frequencies were calculated by direct counting and were compared between patients and controls; etiologic fraction (EF) was calculated according to Olivo-Díaz et al. (2004). Frequencies between groups were compared using Mantel–Haenszel test and Fisher's exact test when appropriate. Odds ratio (OR) and 95% confidence intervals were calculated to estimate the relative risk conferred by a particular allele and genotype using Epi-Info version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA).

## Results

Table 1 summarizes the significant associations found between alleles or genotypes and other comparisons observed in patients with IBS and/or *Blastocystis* or without IBS and/or *Blastocystis* vs. IL-8 and IL-10 SNPs. Five of seven SNPs showed some association with either IBS or *Blastocystis*; thus, polymorphisms for IL-8 at positions +396 and +781, as well as IL-10 at position −1082 were SNPs associated to the development of IBS, while IL-8+781 (T) and IL-10-592 (C) alleles were associated to being a *Blastocystis* carrier, the former in all participants and the latter only IBS group, respectively. No association was found regarding *Blastocystis* and control group, although IL-10-819 (CC) genotype showed some tendency to this regard. Interestingly, participants harboring IL-10-592 (C) allele or IL-8+396(GG) genotype showed an EF >42%, meaning that these variants have a high contribution to the development of IBS in *Blastocystis* carriers; while other alleles and genotypes for IBS or

**Table 1** Association between IBS, *Blastocystis*, and IL-8 and IL-10 gene polymorphisms

| Relationship | Allele or genotype | *p* value[a] | OR (95% CI) | EF |
|---|---|---|---|---|
| IBS vs. control | Allele | | | |
| | IL-8+396 (G) | 0.0437 | 1.78 (1.01–3.12) | 0.291 |
| | IL-10-1082 (A) | 0.0267 | 1.99 (1.08–3.69) | 0.358 |
| | Genotype | | | |
| | IL-8+396(GG) | <0.0001 | 10.13 (2.73–37.55) | 0.313 |
| | IL-8+781(CT) | 0.0248 | 2.45 (1.11–5.39) | 0.348 |
| | IL-10-1082(AA) | 0.0039 | 3.64 (1.49–8.89) | 0.353 |
| Presence or absence of *Blastocystis* | Allele | | | |
| | IL-8+781 (T) | 0.0448 | 2.00 (1.01–3.97) | 0.228 |
| | Genotype | | | |
| | IL-8+396(GG) | 0.0025 | 4.64 (1.63–13.19) | 0.290 |
| IBS and *Blastocystis* carrier vs. IBS and absence of *Blastocystis* | Allele | | | |
| | IL-10-592 (C) | 0.0166 | 3.28 (1.22–8.79) | 0.454 |
| | Genotype | | | |
| | IL-8+396(GG) | 0.0272 | 4.22 (1.15–15.5) | 0.429 |
| Control and *Blastocystis* carrier vs. control and absence of *Blastocystis* | Genotype | | | |
| | IL-10-819 (CC) | 0.0426[b] | 0.12 (0.01–1.05) | – |

*OR (95% CI)* odds ratio (95% confidence interval), *EF* etiologic fraction

[a]Mantel–Haenszel test

[b]Fisher's exact test, two tail

for the presence of *Blastocystis*, showed, independently, low values of EF (<35%).

## Discussion

Despite the fact that *Blastocystis* was discovered almost 100 years ago, its clinical significance and many aspects of its biology remain unresolved. There are arguments for and against its pathogenicity, some being based on parasite loads, genetic type, or subtype and the usefulness of chemotherapeutic intervention (Boorom et al. 2008; Tan 2008; Stensvold et al. 2009b).

The pathophysiology of IBS remains elusive and no single mechanism explains entirely the clinical manifestations of IBS. There are probably several interconnected factors occurring at different level in the patient that consequently may conduce to clinical symptoms of this syndrome (Stark et al. 2007). Thus, some studies focused on the potential role of *Blastocystis* have found this parasite associated to the development of IBS (Yakoob et al. 2004, 2010; Stensvold et al. 2009a; Jimenez-Gonzalez et al. 2011). In addition, SNPs of cytokine genes involved in the regulation of the immune and inflammatory reaction, such as TNF-α, IL-4, IL-6, and IL-10, have been studied in IBS, suggesting that these SNPs might have a role in differential expression, regulating the pathophysiology of the disease (van der Veek et al. 2005; Wang et al. 2006; Barkhordari et al. 2010a, b). However, the concurrent role of IL-8 and IL-10 SNPs and the presence of *Blastocystis* carriers in IBS patients have not been previously evaluated. IL-8 is a cytokine involved in the initiation and amplification of acute inflammatory responses and in chronic inflammation (Modi et al. 1990). In contrast, IL-10 limits and may ultimately cease inflammatory responses (Moore et al. 2001); its production has been associated to some SNPs in the promoter gene region (Turner et al. 1997; Moore et al. 2001).

In the present study, we found that alleles G and A, as well as the homozygous variant for IL-8 at position +396 and IL-10 at position −1082, respectively, were relevant to develop IBS. A case–cohort study performed in a population from North Central China revealed that IL-8+396(T/T) genotype has twofold increased relative risks for gastric adenocarcinoma (Savage et al. 2004), while the same SNPs in Dutch IBS patients were similarly distributed in patients and controls (van der Veek et al. 2005); Gonsalkorale et al. (2003) showed that the high producer IL-10-1082(G) allele and the homozygous variant were slightly higher in healthy controls compared with English IBS patients; and in the present study, we found the low producers IL-10-1082 (A) allele and IL-10-1082(AA) genotype were associated to IBS patients. All these data point to the absence or decrease of IL-10 production in patients, which seems to be logical, since IBS patients have an inflammatory processes and IL-10 is related to regulation of inflammation.

The IL-8+396 (GG) genotype was relevant because it was only found associated to *Blastocystis* carriers and in IBS–*Blastocystis* carrier; in the latter, the influence of the genotype was higher, considering the OR and EF values. IL-8+781 (T) and IL-10-592 (C) alleles were also associated to *Blastocystis* carriers and to IBS–*Blastocystis* carrier, respectively. Puthia et al. (2008) demonstrated that cysteine

proteases of *Blastocystis ratti* induce IL-8 production in colonic epithelial T84 cells and that an NF-κB-dependent transcriptional process is involved. Another study reported that after 24 h incubation of *Blastocystis* with colonic epithelial cells HT-29 and T-84, the production of inflammatory cytokines IL-8 and granulocyte–macrophage colony-stimulating factor, were induced by the presence of this parasite (Long et al. 2001). Besides, in rats infected with *Blastocystis*, a significant upregulation of the expression of interferon-γ, IL-12, and tumor necrosis factor alpha, but not IL-6 or granulocyte–macrophage colony-stimulating factor, was seen in the cecal mucosa at 2 and/or 3 weeks post-infection (Iguchi et al. 2009). Thus, because *Blastocystis* is capable of inducing proinflammatory responses and some SNPs of cytokine genes might have a role in the pathophysiology of IBS, when *Blastocystis* carriers harbor relevant SNPs of IL-8 or IL-10 (particularly for IL-8+396(G) or IL-10-1082 (A) allele), the relative risks for IBS could be >threefold increased. Finally, association between IL-8 and IL-10 gene polymorphisms to the pair IBS–*Blastocystis* carrier suggests either their individual or additive role as relevant factors in the etiology of this disease.

**Acknowledgments** The authors gratefully acknowledge Maria Elena Rodriguez-Campa and Alberto Gonzalez-Angulo for their clinical assistance. Rocio Jimenez-Lucio and David Sierra performed DNA extractions. This work was partially supported by CONACYT grant 69589.

## References

Barkhordari E, Rezaei N, Ansaripour B, Larki P, Alighardashi M, Ahmadi-Ashtiani HR, Mahmoudi M, Keramati MR, Habibollahi P, Bashashati M, Ebrahimi-Daryani N, Amirzargar AA (2010a) Proinflamatory cytokine gene polymorphisms in irritable bowel syndrome. J Clin Immunol 30:74–79

Barkhordari E, Rezaei N, Mahmoudi M, Larki P, Ahmadi-Ashtiani HR, Ansaripour B, Alighardashi M, Bashashati M, Amirzargar AA, Ebrahimi-Daryani N (2010b) T-helper 1, T-helper 2, and T-regulatory cytokines gene polymorphisms in irritable bowel syndrome. Inflammation 33:281–286

Boorom KF, Smith H, Nimri L, Viscogliosi E, Spanakos G, Parkar U, Li LH, Zhou XN, Ok UZ, Leelayoova S, Jones MS (2008) Oh my aching gut: irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. BMC Parasit Vectors 1:40

Fajardo-Dolci G, Solorio-Abreu J, Romero-Alvarez JC, Zavaleta-Villa B, Cerezo-Camacho O, Jiménez-Lucio R, Olivo-Díaz A (2006) DQA1 and DQB1 association and nasal polyposis. Otolaryngol Head Neck Surg 135:243–247

Gonsalkorale WM, Perrey C, Pravica V, Whorwell PJ, Hutchinson IV (2003) Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? Gut 52:91–93

Hotoleanu C, Popp R, Trifa AP, Nedelcu L, Dumitrascu DL (2008) Genetic determination of irritable bowel syndrome. World J Gastroenterol 14:6636–6640

Iguchi A, Yoshikawa H, Yamada M, Kimata I, Arizono N (2009) Expression of interferon gamma and proinflammatory cytokines in the cecal mucosa of rats experimentally infected with *Blastocystis* sp. strain RN94-9. Parasitol Res 105:135–140

Jimenez-Gonzalez DE, Martinez-Flores WA, Reyes-Gordillo J, Ramirez-Miranda ME, Arroyo-Escalante S, Romero-Valdovinos M, Stark D, Souza-Saldivar V, Martinez-Hernandez F, Flisser A, Olivo-Diaz A, Maravilla P (2011) *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. Parasitol Res. doi:10.1007/s00436-011-2626-7

Long HY, Handschack A, König W, Ambrosch A (2001) Blastocystis hominis modulates immune responses and cytokine release in colonic epithelial cells. Parasitol Res 87:1029–1030

Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC (2006) Functional bowel disorders. Gastroenterology 130:1480–1491

Meenagh A, Williams F, Ross OA, Patterson C, Gorodezky C, Hammond M, Leheny WA, Middleton D (2002) Frequency of cytokine polymorphisms in populations from western Europe, Africa, Asia, the Middle East and South America. Hum Immunol 63:1055–1061

Modi WS, Dean M, Seuanez HN, Mukaida N, Matsushima K, O'Brien SJ (1990) Monocyte-derived neutrophil chemotactic factor (MDNCF/IL-8) resides in a gene cluster along with several other members of the platelet factor 4 gene superfamily. Hum Genet 84:185–187

Moore KW, de Waal MR, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765

Olivo-Díaz A, Debaz H, Alaez C, Islas VJ, Pérez-Pérez H, Hobart O, Gorodezky C (2004) Role of HLA class II alleles in susceptibility to and protection from localized cutaneous leishmaniasis. Hum Immunol 65:255–261

Puthia MK, Lu J, Tan KS (2008) *Blastocystis ratti* contains cysteine proteases that mediate interleukin-8 response from human intestinal epithelial cells in an NF-kappa B-dependent manner. Eukaryot Cell 7:435–443

Sambrook J, Fitsch EF, Maniatis T (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor, New York

Savage SA, Abnet CC, Mark SD, Qiao YL, Dong ZW, Dawsey SM, Taylor PR, Chanock SJ (2004) Variants of the IL8 and IL8RB genes and risk for gastric cardia adenocarcinoma and esophageal squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 13:2251–2257

Stark D, van Hal S, Marriott D, Ellis J, Harkness J (2007) Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their diagnosis. Int J Parasitol 37:11–20

Stensvold R, Brillowska-Dabrowska A, Nielsen HV, Arendrup MC (2006) Detection of *Blastocystis hominis* in unpreserved stool specimens by using polymerase chain reaction. J Parasitol 92:1081–1087

Stensvold CR, Lewis HC, Hammerum AM, Porsbo LJ, Nielsen SS, Olsen KE, Arendrup MC, Nielsen HV, Mølbak K (2009a) *Blastocystis*: unravelling potential risk factors and clinical significance of a common but neglected parasite. Epidemiol Infect 137:1655–1663

Stensvold CR, Nielsen HV, Mølbak K, Smith HV (2009b) Pursuing the clinical significance of *Blastocystis*—diagnostic limitations. Trends Parasitol 25:23–29

Tan KS (2008) New insights on classification, identification, and clinical relevance of *Blastocystis spp*. Clin Microbiol Rev 21:639–665

Tan KS, Mirza H, Teo JD, Wu B, Macary PA (2010) Current views on the clinical relevance of *Blastocystis* spp. Curr Infect Dis Rep 12:28–35

Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV (1997) An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 24:1–8

van der Veek PP, van den Berg M, de Kroon YE, Verspaget HW, Masclee AA (2005) Role of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in irritable bowel syndrome. Am J Gastroenterol 100:2510–2516

Wang BM, Jiang XZ, Yang YL, Liu WT, Cao XC, Zhao XZ (2006) A study of interleukin-10 gene polymorphism in irritable bowel syndrome. Zhonghua Nei Ke Za Zhi 45:289–292

Yakoob J, Jafri W, Jafri N, Khan R, Islam M, Beg MA, Zaman V (2004) Irritable bowel syndrome: in search of an etiology: role of *Blastocystis hominis*. AmJTrop Med Hyg 70:383–385

Yakoob J, Jafri W, Beg MA, Abbas Z, Naz S, Islam M, Khan R (2010) *Blastocystis hominis* and *D. fragilis* in patients fulfilling irritable bowel syndrome criteria. Parasitol Res 107:679–684