Investigation of Interleukin-10 Promoter Polymorphisms and Interleukin-10 Levels in Children with Irritable Bowel Syndrome

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Background/Aims: The aim of this study was to investigate whether genetic variations at positions -1082, -819, and -592 in the interleukin (IL)-10 promoter affect IL-10 production in children with irritable bowel syndrome (IBS). Methods: Ninety-four children with IBS and 102 children as healthy controls (HCs) were enrolled. Genomic DNA was extracted, and IL-10 -1082, -819, and -592 polymorphisms were detected by direct sequencing from all participants. Peripheral blood mononuclear cells (PBMCs) from 46 IBS children and 38 HCs were isolated and cultured with and without 5 ng/mL *Escherichia coli* lipopolysaccharide (LPS). IL-10 levels in the culture supernatants were measured by enzyme-linked immunosorbent assay. Results: There were no significant differences in the distribution of IL-10 -1082, -819, and -592 polymorphisms or in the allele and haplotype frequencies between IBS children and HCs. PBMCs from children with IBS had significantly lower IL-10 levels after LPS stimulation than PBMCs from HCs (p=0.011); however, LPS-induced IL-10 levels in PBMCs with different genotypes of -819 and -592 polymorphisms were not significantly different between IBS patients and HCs. Conclusions: Although significantly lower LPS-induced IL-10 production by PBMCs was noted, it is unlikely that IL-10 production was fully genetically determined in our IBS children. ClinicalTrials.gov identifier: NCT01131442.

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Key Words: Irritable bowel syndrome; Child; Interleukin-10; Interleukin-10 gene polymorphisms

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder that mainly affects children >5 years of age and adolescents. It is defined as having abdominal pain, bloating, and changes in bowel habits (e.g., diarrhea, constipation, or mixed) in the absence of any organic or structural abnormality. Various etiologies like visceral hyperalgesia, disturbance of brain-gut interaction, autonomic and hormonal events, genetic and environmental factors, food sensitivity, postinfectious sequelae, and psychosocial disturbances have been implicated, but the precise pathophysiology remains unclear.

Postinfectious IBS introduces a role for the immune activation in the development of IBS symptoms. Postinflammatory changes in the gut may produce chronic alterations of the immune system, and altered cytokine profiles in IBS patients have been shown in some studies. Our previous study also found that children with IBS tend to produce lower amounts of the anti-inflammatory cytokine interleukin (IL)-10 at baseline and after *Escherichia coli* lipopolysaccharide (LPS) stimulation, implying that defects in immune modulation may contribute to IBS in children.

Evidences demonstrate that changes in the genetic make-up or expression of cytokines play a critical role in the inflammatory response in the gut. Although a genetic component is suspected, unambiguous susceptibility genes have not been identified so far. Since the capacity for cytokine production can be genetically determined and is mainly related to genetic variations in the promoter region, further study that measures cytokine profiles and explores cytokine gene polymorphisms in parallel will be beneficial in this field.

Several polymorphic sites within the IL-10 gene promoter region have been described, including three biallelic polymorphisms at positions -1082 (base G to A, db SNP. rs1800896), -819 (base C to T, db SNP. rs1800871), and -592 (base C to A, db SNP. rs1800872).
db SNP, rs 1800872) from the transcription start site, are known to regulate the capacity to produce IL-10. The IL-10 -819 C and T alleles are completely in linkage disequilibrium with the IL-10 -592 C and A alleles, respectively. 11,12

The aim of this study was to investigate if genetic variations at positions -1082, -819, and -592 in the IL-10 promoter affect IL-10 production and predispose to the development of IBS.

MATERIALS AND METHODS

1. Study population

Ninety-four children with IBS (49 females and 45 males; age, 5- to 18-year-old) and 102 healthy children as controls (61 females and 41 males; age, 2- to 18-year-old) were enrolled between November 2008 and February 2011. Patients were recruited consecutively from the outpatient Clinic of the Department of Pediatric Gastroenterology at Chang Gung Memorial Hospitals in Keelung and Taoyuan, whereas healthy volunteers were recruited through advertisements. All of the study participants were Han Chinese.

All patients had chronic or relapsing symptoms of IBS consistent with the Rome II criteria. 7 The symptoms were present for at least 3 months. Patients were further categorized based on their symptoms and predominant stool patterns. 11 Patients with more than three bowel movements per day and loose/watery stool consistency were categorized as diarrhea-predominant IBS (D-IBS, n=32), while those with fewer than three bowel movements per week and hard or lumpy stools were categorized as constipation-predominant IBS (C-IBS, n=33) and those with an alternating bowel pattern were categorized as mixed IBS (M-IBS, n=29).

A comprehensive diagnostic work-up, including hemogram, biochemistry, abdominal sonography, and serial stool testing, were conducted to exclude acute infections or any evidence of structural anomaly that may cause the symptoms.

The hospital’s Human Ethics Committee (Institutional Review Board) approved the study and all of the participants provided informed consent.

2. DNA extraction

Genomic DNA was extracted from a 1 mL sample of whole blood from 94 IBS patients and 102 healthy controls and collected into tripotassium ethylenediaminetetraacetic acid sterile tubes. Extraction was performed using QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer’s instructions.

3. IL-10 genotyping

The three biallelic IL-10 promoter polymorphisms (-1082, -819, and -592) were detected by polymerase chain reaction (PCR) using common primers (forward, 5’-ATC CAA GAC AAC ACT ACT AA-3’; reverse, 5’-TAA ATA TCC TCA AAG TTC C-3’). These primers yielded an amplicon 587 bp in size (-1115 to -528) containing the above polymorphisms. The amplification process was performed in 20 µL containing 1 µL of template DNA, PCR master mix 10 µL (RBC SensiZyme® HotstartTaq Premix; RBC Bioscience, Taipei, Taiwan), MgCl₂ (2.5 mmol/L) 1 µL, each primer 2 µL, and free water 4 µL. The parameters for thermocycling were as follows: denaturation at 95°C for 10 minutes, followed by 30 denaturation cycles at 95°C for 30 seconds; annealing at 55.5°C for 30 seconds; and extension at 72°C for 1 minute. This was followed by a final extension at 72°C for 5 minutes and then storage at 4°C. After confirming the final products by electrophoresis on agarose gels (%), all of the PCR products were sequenced using ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The nucleotide polymorphisms at positions -1082, -819, and -592 were read directly.

4. Isolation of peripheral blood mononuclear cells

Blood samples (8 to 10 mL) from 46 (D-IBS, n=20; C-IBS, n=12; and M-IBS, n=14) of the 94 IBS children and 38 of the 102 healthy controls (HCs) were taken upon enrollment and those who used probiotics, antibiotics, analgesics, or immunosuppressive drugs within the past month were not included. In addition, subjects with recent infections, major allergic diseases, food intolerance, or psychiatric disorders were excluded. Peripheral blood mononuclear cells (PBMCs) were isolated from the buffy coats on Lymphoprep (Nycomed, Oslo, Norway) gradients by centrifugation. After washing, the cells were resuspended at a concentration of 1×10⁶ cells/mL in Roswell Park Memorial Institute 1640 medium containing 10% heat-inactivated fetal bovine serum.

5. Cell cultures

The concentration of PBMCs was adjusted to 10³ cells/mL in complete medium and then transferred to 24-well plates. Some were stimulated with E. coli LPS 5 ng/mL (SIGMA L4391; Sigma, St. Louis, MO, USA), while other were not. Duplicate cultures were prepared and incubated for 24 hours at 37°C in a humidified 5% CO₂ atmosphere. The supernatants were collected, pooled, and stored at -20°C until analysis by enzyme-linked immunosorbent assay (ELISA).

6. ELISA

Concentrations of IL-10 in the culture supernatants were determined using commercially available kits according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA: IL-10, D1000B). Optical density was measured at wavelength of 450 nm and reference wavelength of 590 nm. The values were correlated linearly with cytokine standards. The sensitivity limit of the assay was 5 pg/mL.

7. Statistical analysis

Genotype and allele frequencies of each IL-10 polymorphism