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ORIGINAL ARTICLE

Interleukin-23p19 expression in patients with ulcerative colitis and its relation to disease severity

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Interleukin 23p19 gene;
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Summary *Background:* The purpose of this study was to determine whether the mucosal expression of interleukin (IL)-23p19 has a role in the pathogenesis of ulcerative colitis, and to determine its relation to disease severity.
Methods: This study was performed on 50 patients with ulcerative colitis and 10 normal individuals as the controls. They were divided into Group I (27 patients with mild to moderate disease), Group II (23 patients with severe disease), and Group III (10 normal individuals). All patients and the controls were subjected to histopathological study, IL-23p19 immunohistochemical staining, IL-23R expression by flow cytometry, and serum IL-23 by enzyme-linked immunoassay.
Results: There was a significant increase in IL-23p19 gene expression and IL-23R level in patients with ulcerative colitis, compared to the controls. A significant positive correlation was detected between increased expression of the IL-23p19 gene, IL-23R, high serum IL-23, and the severity of the disease.
Conclusion: Increased expression of the IL-23p19 gene has a role in the pathogenesis of ulcerative colitis. Targeted therapy directed against IL-23p19 may be effective in its treatment. Increased expression of the IL-23p19 gene and IL-23R with high serum IL-23 is correlated positively with disease severity.

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Introduction

Inflammatory bowel disease consists of two distinct diseases: Crohn's disease (CD) and ulcerative colitis (UC). Both diseases may arise because of the combination of genetic variations and alterations in bacterial flora that can subsequently drive a dysregulated immune response that results in chronic intestinal inflammation [1,2].

Interleukin (IL)-23 is a member of a small family of proinflammatory cytokines, consisting of a p19 subunit and a common p40 subunit that is shared with IL12 [3]. The receptor for IL-23 (IL-23R) consists of the IL-12R beta 1 subunit and a novel component termed "IL-23R" [4], which is expressed predominantly on T cells, natural killer (NK) cells, and natural killer T (NKT) cells, and to a smaller extent on monocytes, macrophages, and dendritic cells (DCs) [5]. Interleukin 23 has a crucial role in the pathogenesis of several immune-mediated inflammatory diseases by recruiting several inflammatory cells and Th17 cells [6,7]. Interleukin 23 promotes Th17 cells in producing tumor necrosis factor alpha (TNFα), IL-17, IL-6, IL-22, granulocyte-macrophage colony-stimulating factor, and other novel factors, which are associated with the induction of autoimmune inflammation [6,8,9]. This study was performed to determine whether the mucosal expression of IL-23p19 has a role in the pathogenesis of UC and to elucidate its relation to disease severity.

Materials and methods

Patients and methods

This study was performed on 50 patients with UC and 10 individuals (i.e., the controls) for whom colonoscopic and histopathologic findings were normal. This study was accepted by the medical ethics committee of the Faculty of Medicine at Tanta University (Tanta, Egypt). Each patient provided written consent after they received a complete explanation of the protocol of the study. The studied patients were selected from the inpatient and outpatient clinics of the Tropical Medicine and Internal Medicine Departments of the Tanta University Hospital (Tanta, Egypt) in the period between November 2012 and October 2013. Ulcerative colitis patients were diagnosed on the basis of clinical, endoscopic, and histological manifestations according to the criteria of the American Gastroenterology Association [10]. The patients were divided, based on endoscopic and histopathological findings, into Group I (27 patients with mild to moderate UC disease), Group II (23 patients with severe UC disease), and Group III (10 individuals whose colonoscopic and histopathologic findings were normal as control). Patients in the following categories were excluded from the study: patients who were pregnant; patients who had a malignancy, heart failure, renal failure, thyroid disorders, acute infection, or stroke; and patients taking immunosuppressive drugs.

All patients and controls were subjected to a complete history taking and thorough clinical examination. Laboratory investigations such as complete blood picture, blood urea and serum creatinine, erythrocyte sedimentation rate, and stool examination were performed to exclude bacterial causes of colitis. Colonoscopy was performed in all groups and the severity of the disease was determined. The endoscopic scoring system for UC by Pineton de Chambrun et al [11] was used, as follows: Score 0, normal or inactive disease; Score 1, mild disease (i.e., erythema, decreased vascular pattern, and mild friability); Score 2, moderate disease (marked erythema, increased vascular pattern, friability, and erosion); and Score 3 (severe disease with spontaneous bleeding and ulceration). Endoscopic findings were recorded and multiple biopsies were obtained for histopathology, IL-23p19 immunohistochemical staining, and IL-23 R expression by flow cytometry.

Histopathological study

Four-micrometer thick serial sections of formalin fixed, paraffin-embedded tissue were cut and stained using hematoxylin and eosin for histopathological evaluation and grading of the groups. A six grade classification system for inflammation was used. The grades were: 0, structural change only; 1, chronic inflammation; 2, lamina propria neutrophils; 3, neutrophils in the epithelium; 4, crypt destruction; and 5, erosions or ulcers [12].

Interleukin-23p19 immunohistochemical staining

Four-micrometer thick serial sections of formalin fixed, paraffin-embedded tissue were cut and mounted on positively charged glass slides. After incubation at 60°C overnight and deparaffinization, the sections were placed in 0.01M sodium citrate buffer (pH 6.0) and heated twice for 5 minutes in a microwave oven. After inactivation of endogenous peroxidase with 0.5% metaperiodic acid in phosphate-buffered saline (PBS) for 10 minutes, the sections were incubated with 10% horse serum in PBS for 1 hour. The sections were then incubated at 4°C overnight with 100× diluted primary goat antimouse IL-23p19 antibody (R&D Systems, Inc., Minneapolis, MN, USA). The standard avidin—biotinperoxidase complex (ABC) technique was performed using the LabVision Secondary Detection Kit (UltraVision Detection System Anti-polyvalent, HRP). The color was visualized by incubation with chromogen 3,3′-diaminobenzidine for 5 minutes. The slides were then counterstained with Mayer hematoxylin and cover slipped with Permount (StatLab, McKinney, TX). Negative controls without the primary antibodies were set for each test.

Immunohistochemical evaluation

Results were expressed semiquantitatively. Positively stained cells were counted by examining at least 10 random fields (200×) in each section and expressed as the percentage of positive cells over the total cell number [13].

IL-23R expression by flow cytometry

Peripheral blood mononuclear cells

Lymphocytes were isolated from peripheral blood by incubation with Rosette Sep Human CD4+T cells (cytotoxic T-lymphocytes) enrichment cocktail (Stem Cells Technologies, Grenoble, France), followed by centrifugation on a