A Th1 but not a Th17 response is present in the gastrointestinal involvement of Behçet's disease

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ABSTRACT

Objectives. *Behçet's disease has been historically classified as a Th1 disease. The recently described IL-17/IL-23 pathway seems to play an important role in many inflammatory diseases and in the intestinal abnormalities of AS and CD. The aim of the present study was to evaluate the IL-17/IL-23 axis in parallel with Th1 and IL-27 response in the intestine of patients with BD and gastrointestinal abnormalities.*

Methods. *Quantitative TaqMan reverse transcriptase-polymerase chain reaction (RT-PCR) was utilised for all determinations on ileal biopsy specimens obtained from BD, AS and CD patients. The serum levels of Th1 and Th17 cytokines were evaluated by enzyme-linked immunosorbent assay.*

Results. *A Th1 but not a Th17 response is present in the gastrointestinal involvement of Behçet's disease.*

Conclusions. *Although BD shares clinical manifestations with both CD and AS, the immunologic abnormalities seen in the intestine are quite different, indicating that other immune mechanisms should be taken into account.*

Introduction

Behçet's disease (BD) is a rare vasculitis characterised by recurrent oral and genital ulcers, eye lesions, skin lesions and positive pathergy test. Oral ulcers, erythema nodosum, uveitis and arthritis are common manifestations of BD together with intestinal inflammation but also of ankylosing spondylitis (AS) and Crohn's disease (CD).

We have recently produced evidence that AS and CD share some clinical and immunological similarities at gastrointestinal level (1).

Activation of T cells and monocytes/macrophages is regarded as an important factor in the pathogenesis of BD as well as AS and CD. Historically BD has been considered to be a typical Th1 disease, characterised by increased levels of Th1 cytokines such as IFN-γ, IL-2 and TNF-α (2, 3).

The IL-17/IL-23 pathway seems to play an important role in many inflammatory diseases (4) and in the intestinal abnormalities of AS and CD (1). Increased IL-17 levels have been found in the sera of BD patients (5), it is not yet clear, however, whether the IL-17/IL-23 pathway is involved in the pathogenesis of the intestinal BD. Data available, in fact, on cytokine production in the gut of BD patients are limited and indicate a small impairment of Th1 cytokines (6).

The aim of the present study was to evaluate the IL-17/IL-23 axis in parallel with Th1 response in the intestine of patients with BD and gastrointestinal abnormalities.

Patients and methods

Intestinal biopsy specimens were obtained, after informed consent, from 11 patients (7 males and 4 females, age range 25–58 years) with BD and intestinal complaints. Two adjacent mucosal biopsy samples from the ileum were obtained from each subject, independently of the presence of macroscopic involvement. All patients fulfilled the international criteria for disease classification and were considered to have an active disease (7). Ileal specimens from 10 healthy subjects, 8 AS and 7 CD patients who underwent ileocolonscopy for routine clinical evaluation were also included as healthy and disease controls, respectively. The AS group consisted of 5 men and 3 women with a median age of 41 years (range 38–65 years) who were diagnosed as having AS according to the modified New York criteria (8). Disease activity was evaluated using the Bath AS Disease Activity Index (BASDAI) (9), with a

BASDAI score of >4 indicating active disease. All patients were HLA–B27 positive. At the time mucosal biopsy specimens were obtained, the mean ± SD BASDAI score was 6.7±3.4. The CD group consisted of 5 men and 2 women with a median age of 50 years (range 48–60 years). The diagnosis of CD was made according to clinical parameters and findings of radiographic, endoscopic, and histopathologic analyses. Disease activity was evaluated using the CD Activity Index (10), with a score >150 indicating active disease, and endoscopic and histopathologic data. At the time of sample collection, the mean ± SD CD Activity Index score was 253±51.5, and none of the patients had received steroids and/or cytotoxic drugs, immunosuppressive agents, or antibiotics. The control group (NHS) consisted of 6 men and 4 women with a median age of 45 years (range 41–58 years) who were undergoing ileocolonoscopy for diagnostic purposes. The study was approved by the ethics committee and the institutional review board of the University of Palermo.

The quantitative TaqMan reverse trascriptase-polymerase chain reaction (RT-PCR) was utilised for all determinations as extensively described in reference 1. Total RNA was extracted using the Qiagen RNeasy Mini kit (Qiagen, Chatsworth, CA), with on-column DNase I digestion. A total of 1 μg of RNA was reverse-transcribed to complementary DNA (cDNA) using a ThermoScript First-Strand cDNA Synthesis kit (Invitrogen, Carlsbad, CA). Samples were run in triplicate at 20 ng of cDNA per well and detected using an ABI Prism 7900HT instrument. Results were analysed using ABI Prism 7900HT Sequence Detection System version 2.1 software. Relative quantification was assessed using the Ct method.

IL-12, IL-17, IFN-γ, TNF-α serum levels were analysed with Human Autoimmune Response Multi-Analyte ELISArray kits (SABiosciences Corp, Frederick, Md) according to the manufacturer's instructions. IL-23 and IL-27 serum levels were evaluated using commercially available kits (R&D Systems, Minneapolis, MN).

Statistics

Results are expressed as mean ± SD. Statistical analysis of quantitative variables was performed using the Kruskal-Wallis nonparametric test, with Dunn's post test when analysing more than 2 groups and the Mann-Whitney U-test when analysing 2 groups. *P*-values less than 0.05 were considered significant.

Results

Six patients complain abdominal pain, 4 diarrhoea and one both. At morphol-

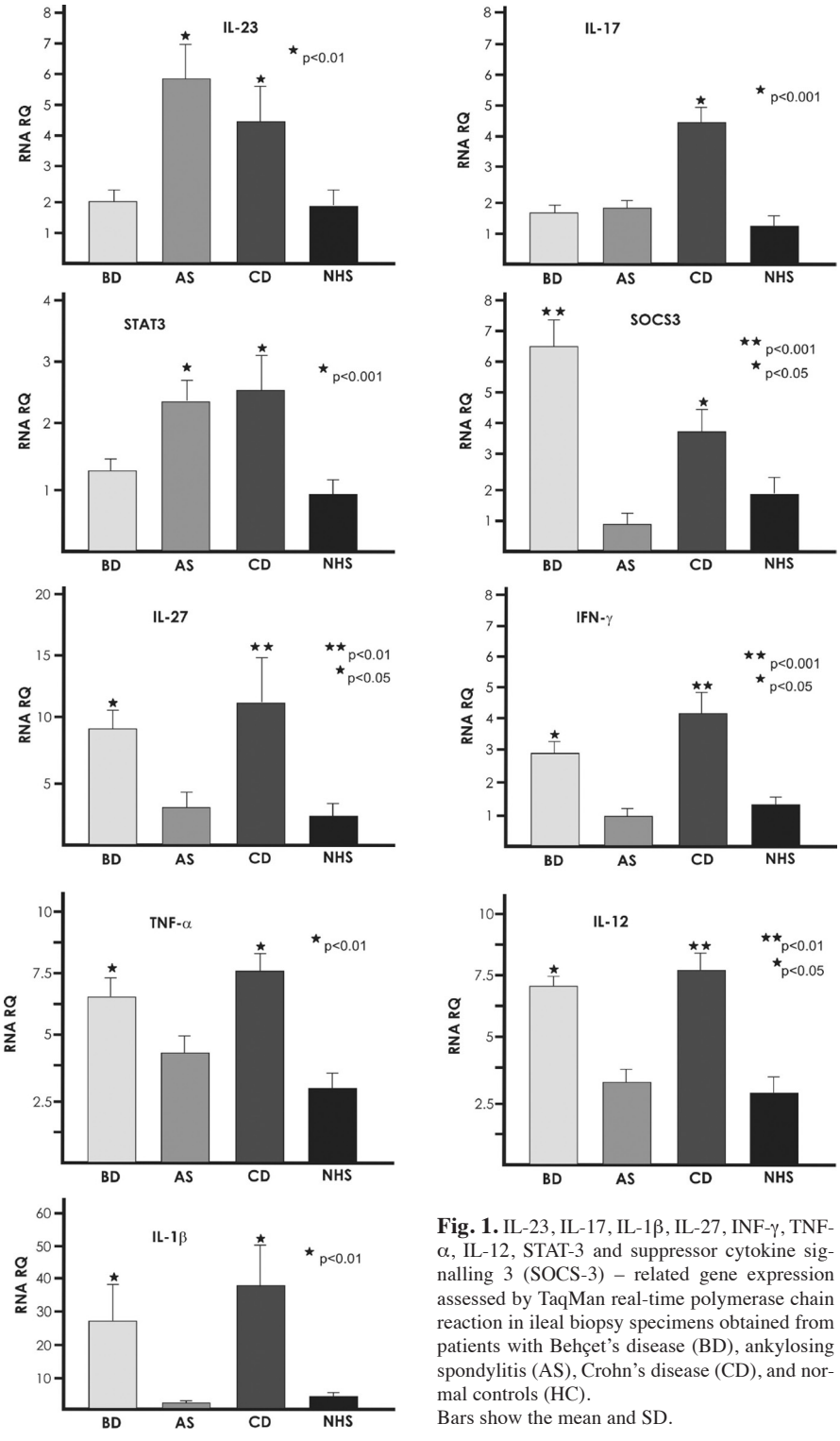

Fig. 1. IL-23, IL-17, IL-1β, IL-27, INF-γ, TNF-α, IL-12, STAT-3 and suppressor cytokine signalling 3 (SOCS-3) – related gene expression assessed by TaqMan real-time polymerase chain reaction in ileal biopsy specimens obtained from patients with Behçet's disease (BD), ankylosing spondylitis (AS), Crohn's disease (CD), and normal controls (HC).
Bars show the mean and SD.

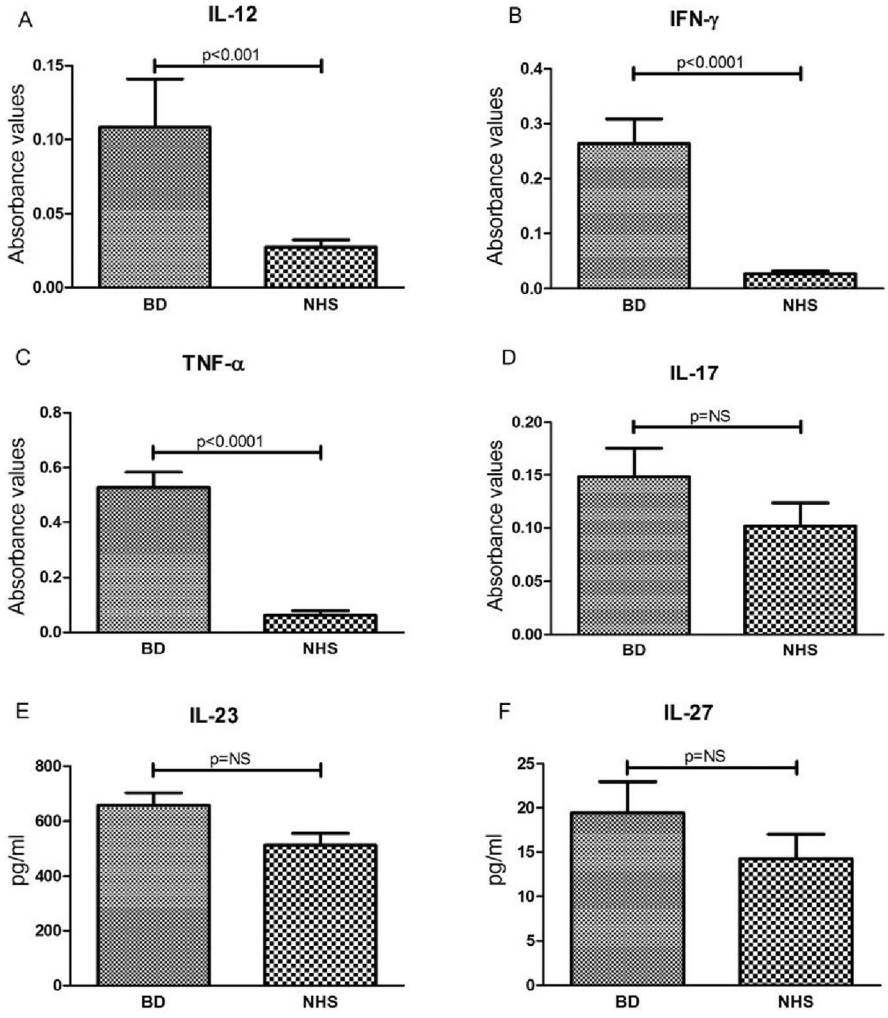

Fig. 2. IL-12 (A), IFN-γ (B), TNF-α (C), IL-17 (D) IL-23 (E), and IL-27 (F) were measured in serum samples from patients with Behçet's disease (BD) and healthy controls (HC) by ELISArrays. A-D data are presented as absorbance values at 450 nm. IL-23 and IL-27 levels are expressed as pg/ml.

ogy patients showed chronic non-specific inflammatory abnormalities and in some cases venule vasculitis and apthous ulcers. Patients were untreated (n=3) or treated with colchicine (n=4) low dose prednisone (n=2) or both (n=2). None was under immunosuppressants. All CD patients had an active disease. Of the 8 patients with active AS who underwent colonoscopy (mean BASDAI score 6.7), evidence of subclinical intestinal inflammation was observed in 5 (one with acute inflammation and 4 with chronic inflammation). Results are shown in Figure 1. In contrast with results obtained in AS and CD patients, normal levels of IL-23 mRNA were found in BD. STAT-3, which mediates IL-23 signalling upon IL-23 receptor ligation, and IL-17A mRNA were also normal. Samples from BD patients displayed SOCS-3 over expression at levels comparable to those observed in CD but not in AS patients. Levels of TNF-α, IFN-γ, IL-12p35 mRNA in BD specimens were significantly higher than those of healthy controls and AS and comparable to those found in CD. Interestingly IL-27, a Th1 cytokine which inhibits *de novo* Th17 development from naive T cells (11) was found to be markedly up-regulated at levels similar to those found in CD.

Analysis of serum cytokines showed increased concentrations of IL-12, TNF-α and IFN-γ in BD patients when compared to NHS (Fig. 2). A trend toward an increase in IL-17, IL-23 and IL-27, (although not statistically significant) was observed in BD patients versus NHS.

Discussion

The presence of a shared clinical behaviour between BD, AS and CD is suggested by evidence of similar arthropathy, intestinal inflammation and muco-cutaneous abnormalities in patients with either disease.

Based on recent findings, it is becoming increasingly clear that IL-23 exerts an essential pathogenetic role in promoting autoimmunity and chronic inflammation in several models of autoimmune diseases, such as experimental inflammatory arthritis (12) and experimental colitis (13). In this and our previous study (1) we have demonstrated a strong and significant up-regulation of IL-23p19 transcripts in the terminal ileum of patients with AS and patients with CD. Unlikely CD and AS, in BD patients IL-23 was not up-regulated and there was no involvement of the IL-17/IL-23 pathway. On the other hand, intestinal BD seems to be characterised by a Th1 response. Levels of TNF-α, IFN-γ, IL-12p35 mRNA were, in fact, significantly higher than those of healthy controls and AS and comparable to those found in CD. IL-27 was found also markedly up-regulated and may account for the limited Th-17 cell pool in the BD intestine (11). Analysis of serum cytokines concentration by ELISArrays showed a marked and significant increase of Th1, but not Th17 cytokines in BD patients when compared to NHS. The Th1 prevalent response in BD could be related to a defective regulatory T cell response as suggested by a recent study showing that TGF-beta1 and IL-10 gene polymorphism may affect host susceptibility to BD (14).

At present we do not know whether mRNA levels found in our patients are paralleled by their corresponding protein levels. However, the BD population in this study was not large enough to consent definite conclusions. Some considerations, however, may be done. In particular, although BD shares clinical manifestations with both CD and AS, the immunologic abnormalities seen in the intestine are quite different indicating that other immune mechanisms, possibly involving gamma/delta T cells (15), should be taken into account.

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