Cytokine 92 (2017) 12–19

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Alterations in cytokine gene expression profile in colon mucosa of Inflammatory Bowel Disease patients on different therapeutic regimens

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ARTICLE INFO

Article history:
Received 5 June 2016
Received in revised form 7 January 2017
Accepted 9 January 2017

Keywords:
Inflammatory Bowel Disease
Cytokines
IL-17
FoxP3
Th17
Tregs
Immunosuppressive therapy

ABSTRACT

Inflammatory bowel disease (IBD) is assumed to be caused by genetic and environmental factors that interact together in promoting intestinal immune dysregulation where cytokines have validated role. However, the underlying intimate mechanisms in the human IBD involving cytokines still needs to be supplemented especially in the clinical context. The aim of this study was to investigate the expression of some inflammatory and regulatory cytokines (IL-17A, IL-23, IL-6, TGFβ1, and IL-10) as well as of the transcription factor FoxP3 in mucosal samples of IBD and non-IBD patients. We assessed the mRNA relative quantities (RQ) of the above-mentioned cytokines and the transcription factor FoxP3 in paired colonic samples (inflamed and adjacent normal mucosa) from 37 patients with IBD and in normal mucosal tissue in 12 persons without IBD by performing a qRT-PCR assay and tested the protein levels of target cytokines in serum samples. The patients were divided into three groups: without any therapy (n = 10), on 5-ASA (n = 11) and on immunosuppressants (Azathioprine ± 5-ASA/corticosteroids) (n = 16) in order to compare the RQ values for each therapeutic group.

All investigated genes were found upregulated in the inflamed mucosa of IBD patients in the following order: IL-6 > FoxP3 > TGFβ1 > IL-23 > IL-17A > IL-10. We also observed that the gene expression of FoxP3 and IL-6 were substantially higher in the inflamed mucosal tissue of the IBD patients than the adjacent normal mucosa ($p = 0.035$, $p = 0.03$ respectively). Differences between higher mRNA expression of FoxP3 and IL-6 in inflamed tissue were considered significant in patients with ulcerative colitis (UC) ($p = 0.011$, $p = 0.000$ respectively) and with Crohn's disease (CD) ($p = 0.008$, $p = 0.000$ respectively) in comparison to the normal mucosa of non-IBD persons and we found increased TGFβ1 in CD patients alone ($p = 0.041$). Furthermore, IL-6 and TGFβ1 were overexpressed (RQ > 10) in non-inflamed mucosa from IBD patients compared to the normal mucosa from the controls. When we compared the gene expression for paired mucosa in the immunosuppressive treated group with the 5-ASA treated group we observed opposite changes in IL-6 and TGFβ1 expression. Additionally, we found higher serum levels of IL-23 ($p = 0.008$), TGFβ1 and IL-6 in IBD patients compared to non-IBD patients. The obtained specific expression profile consisting of IL-6, TGFβ1, IL-10 and FoxP3 may represent a transcriptional hallmark for IBD. Furthermore, we found that treatment with immunosuppressive therapy was more beneficial for driving cytokine expression to restore immune regulation in patients with IBD, unlike the 5-ASA therapy.

1. Introduction

Inflammatory bowel disease (IBD) is assumed to be caused by genetic and environmental factors that interact together in promoting intestinal immune dysregulation [24,44]. Both entities classically referred as IBD – ulcerative colitis (UC) and Crohn's disease (CD), are examples of complex disorders, which may possess inflammatory and autoimmune features [9]. Both innate and

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http://dx.doi.org/10.1016/j.cyto.2017.01.008
1043-4666/

adaptive immune cells have been identified as the key participants in IBD. The innate immune system takes part mainly at the very outset of an inflammatory response, whereas the adaptive immune system maintains the inflammation [11]. The inflamed mucosa in IBD patients is heavily infiltrated with distinct T-helper cells: Th1, Th2 [14] and also Th17 cells [6,17], which lymphocytes produce a various array of cytokines [13]. Cytokines are the crucial mediators of cells dynamic interplay [24,44], but they are also key regulators of naïve T cells differentiation. Th17 cells express the transcriptional factors RORγt and RORα only in the simultaneous presence of two cytokines-transforming growth factor β-1 (TGFβ1) and IL-6 [2] and are characterized by a certain cytokine profile (17A, 17F, 17AF), IL-21, IL-22 and IL-26 [3,27], generally found to be proinflammatory [6,16]. They strongly perpetuate the intestinal inflammation during IBD. Meanwhile, Th17 cells play an important role at mucosal interfaces in the defense against bacteria and fungi [36]. The maintenance and expansion of Th17 cells depend also on the presence of IL-23, a heterodimeric cytokine which shares its p40 subunit with IL-12 [2,30,35]. Moreover, the IL-23/Th17 pathway has been proposed to contribute to local chronic inflammation in intestines, whereas the IL-12/Th1 pathway is possibly connected with the systemic inflammation in IBD patients [43,46].

Meanwhile, there is some evidence for another regulatory role of Th17 cells [19] – in the absence of IL-23 they can produce IL-10 and this mechanism may play negative feedback control of the inflammation [16].

The function of regulatory T lymphocytes (Tregs), phenotypically described as CD4 + CD25+/-FoxP3+ is to drive the suppressive immune response. However, their role in IBD is not completely understood. Naturally occurring Tregs (nTregs) are generated if TGFβ1 is predominantly expressed and inducible Tregs (iTergs) derived from the differentiation of naïve T cells, named as nTregs, stimulated by IL-10, TGFβ1, and vitamin D3 in the periphery [18]. It has been suggested that Tregs interact with dendritic cells and consequently suppress the immune response in the mucosa, including the pathogenic effects of IL-17 – producing cells through IL-10 [18]. Tregs capable of producing IL-17 have been also described in IBD and colon cancer [28].

Although the pathophysiology of both CD and UC is better understood, the underlying intimate mechanisms in the human IBD involving cytokines still needs to be supplemented especially in the clinical context [15,24]. IBD – related tissue damages are determined by an integrated response of the innate, adaptive and regulatory immune response against intestinal challenges [10]. Since there are strong proofs that the inflammatory processes take part in the intestinal mucosal damages, the cytokines are the main source of biomarkers in IBD [10]. The quantitative real-time polymerase chain reaction (qRT-PCR) could be successfully employed in the gene expression analyses of IBD-related cytokines, chemokines, adhesion molecules and their respective receptors, revealing their participation in the pro- and anti-inflammatory processes [10,24,32,45]. The involvement of cytokines in the intestinal inflammation of IBD is intensively studied recently and their implication has been proven in experimental animal models [24,44]. Specific gene expression profile, represented the molecular events in the inflamed mucosa of IBD patients, could be of benefit as a future diagnostic and a follow-up tools for these patients. Lack of adequate investigations in humans designates for the need for predictive and prognostic markers that can be easily obtained from the patients samples and based on the molecular fingerprinting technologies of IBD in every single patient [10]. For this reason we decided to design our study by firstly investigating a panel of cytokines related to Th17 and Tregs, as well as the transcription factor FoxP3, in order to specify the gene and protein expression profile in inflamed and in the adjacent visibly normal colonic samples obtained from Bulgarian IBD patients. Of particular interest for us was to discover if there were differences in gene expression profile in patients with UC in contrast to CD patients. We also questioned whether the type of therapy was able to change the gene expression profile and if it was true in what direction. To address these questions, we purposed to evaluate specific cytokine expression profiles by examining mRNA and protein expression of the cytokines IL-17A, IL-23, IL-6, TGFβ1, and IL-10 and the transcription factor FoxP3 in paired colonic mucosa samples of IBD patients and to compare them with the profiles obtained from the control group of non-IBD patients. We also assigned a goal to investigate the differences in mRNA expression profile in IBD patients on different therapeutic regimens and to compare the protein levels of the target cytokines of the IBD and non-IBD patients' sera.

2. Material and methods

2.1. Subjects

We investigated colonoscopically obtained biopsy samples of paired mucosal samples (inflamed and adjacent normal tissue) from 37 patients with IBD (23 with UC and 14 with CD) at mean age 40 ± 16 years. An overwhelming proportion of the patients (30/37) was in a state of activity which was assessed by Mayo Clinic score of activity for UC patients and by Crohn's disease activity Index (CDAI) for CD patients. The biopsies were taken during a routine colonoscopy after written confirmed consent signed as following: 3 samples from inflamed and 3 samples from adjacent normal mucosa from each study patient, where the total number of biopsies was 6.

Patients with IBD were divided into three groups: without any therapy (n = 10), on 5-ASA (5-aminosalycilates) alone (n = 11) and on immunosuppressive drugs (Azathioprine ± 5-ASA/corticosteroids) (n = 16). The patients without any therapy were mostly newly diagnosed, whereas the patients on therapy were adherents to the standard weight-adjusted dose of 5-ASA or Azathioprine ± 5-ASA/corticosteroids for a minimum of 3 months. The descriptive statistics of the IBD patients is shown in Table 1. The control group included 12 age and sex matched persons without IBD. They were diagnosed with chronic colitis or anemia during endoscopic and histological evaluation.

All patients and control subjects were found negative for autoimmune disease markers (such as anti-nuclear antibodies, rheumatoid factor, anti-neutrophil cytoplasmic antibodies). The study was approved by the Ethic Committee of the Medical University of Sofia and University Hospital St. Ivan Rilski. All patients were informed about the purpose of the study.

Table 1
Descriptive characteristics of IBD patients.

Characteristics	Number (%) or Mean ± SD
Number	37
UC patients	23 (62%)
CD patients	14 (38%)
Age (years)	40 ± 16
Female	20 (54%)
Activity of the disease	
State of activity	30 (81%)
State of remission	7 (19%)
Therapy	
Without	10 (27%)
On 5-aminosalycilstes (5-ASA)	11 (29.8%)
On 5-ASA ± Immunosuppressants	16 (43.2%)