Serum concentration of interleukin 10, anti-mannan Candida antibodies and the fungal colonization of the gastrointestinal tract in patients with ulcerative colitis

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ABSTRACT

Purpose: There is evidence for the immunomodulation disorders in the response to intestinal flora in inflammatory bowel disease, however, the role of yeasts in the aetio-pathogenesis of ulcerative colitis has not been fully clarified. The aim of this study was to assess the serum concentration of interleukin 10 (IL-10), serum levels of anti-mannan Candida antibodies and fungal colonization of the lower part of the gastrointestinal tract in accordance with the clinical course of ulcerative colitis.

Material/Methods: In 42 consecutive patients with ulcerative colitis serum concentration of IL-10 and anti-mannan Candida antibodies serum levels were measured with ELISA and the quantitative and qualitative fungal cultures of stool samples were performed.

Results: In 20 patients IL-10 serum concentration was below the test sensitivity and in 11 patients it ranged between 0.78 and 9.43 (mean 3.38 +/- 2.8) pg/mL. Anti-mannan Candida antibodies were detected in 8 subjects (19.04%). Stool cultures revealed significant fungal colonization in 3 (8.33%) patients with the predominance of Candida albicans. In comparison with mild/moderate UC, IL-10 serum concentration was not higher in patients with severe course of the disease.

Conclusions: The results of our study show that IL-10 serum concentration correlates neither with the disease activity nor with the levels of anti-mannan Candida antibodies and the fungal colonization of the gastrointestinal tract in ulcerative colitis. It seems that IL-10 serum concentration cannot be a universal marker for the assessment of ulcerative colitis activity. Moreover, anti-mannan Candida antibodies and significant fungal colonization are present in the minority of patients with ulcerative colitis suggesting that yeasts have minor, if any, influence on the clinical course of the disease.

Key words: anti-mannan antibody, fungal colonization, interleukin 10, ulcerative colitis

INTRODUCTION

Crohn’s disease (CD) and ulcerative colitis (UC) are two clinically different types of inflammatory bowel disease (IBD) characterized by chronic, recurrent and tissue damaging inflammation of the gastrointestinal (GI) tract. Although the aetiology and pathogenesis of IBD are not fully understood, there is evidence for immunomodulation disorders of the gut in response to intestinal flora. Cytokines are essential mediators of inflammation in IBD as well as targets for novel therapeutic options, including biological treatment [1,2]. Disturbed balance between proinflammatory and anti-inflammatory cytokines promotes long-standing active inflammation and subsequent tissue injury [3]. Interleukin 10 (IL-10) is an immunomodulatory molecule released by Th2 lymphocytes, which inhibits the production of proinflammatory cytokines involved in the pathogenesis of IBD [4,5]. IL-10 production remains under genetic control and the genotype determines its high or low synthesis in health and disease [6,7]. It has been suggested that genetic predisposition to lower production of IL-10 may increase the risk of IBD, especially UC [7-9]. Interleukin 10–deficient mice develop chronic noninfectious intestinal inflammation [10]. This finding corroborates the action of IL-10 against chronic stimulation of the gut immune...
system, although it seems that colonic activity of IL-10 is insufficient to control inflammation in UC. It has been demonstrated that an elevated production of proinflammatory cytokines by IBD mononuclear cells could be downregulated in vitro by exogenous IL-10 and also in vivo with topical IL-10 enema preparations [11]. However, clinical value of IL-10 measurements in patients with UC remains unclear.

The idea that microorganisms play a role in the aetiology of IBD has gained ground considerably in recent years. The large number of proposed infectious agents may partly be explained by alterations caused by these microorganisms to the normal intestinal flora. Thus extensive experimental and clinical data suggest that luminal bacteria or bacterial products play a significant role in the initiation and perpetuation of chronic intestinal inflammation [12]. Until recently microbiological studies have been focused on bacterial aspects in the pathogenesis of IBD, however, an interest in the presence of fungi in the GI tract has also increased lately. Nevertheless, the number of clinical trials dealing with fungi is relatively scanty in comparison with the research on bacterial pathogens in UC.

Fungi, mostly Candida spp., despite representing only a small fraction of the physiological human GI tract microbiota, are also considered as a risk factor of opportunistic infections in immunocompromised patients. Mechanisms of bacterial and fungal balance, still explored to a small extent, may play an important role in the pathogenesis of UC. Candida spp. are supposed to facilitate permeation of food antigens through the mucosal barrier of the GI tract with the involvement of mast cells via mediators like protease II or TNF-α [13]. The antibacterial and immunosuppressive therapy often used in the treatment of UC may favour yeasts’ multiplication and development of mycoses, especially in the presence of superficial mucosal lesions of the colon.

The anti-mannan Candida antibodies (AMCA) against the mannann component of Candida albicans (C. albicans) cell wall are present in 76% of healthy adults [14]. The association between the elevated level of AMCA and the clinical development of candidiasis has been observed in some groups of patients [15]. AMCA and the assessment of fungal colonization of the GI tract might be useful in better understanding of aetiopathogenesis of UC.

Considering these facts, the aim of the study was to assess IL-10 serum concentration and AMCA serum levels as well as fungal colonization of the lower part of the GI tract in adult patients with UC and their possible correlation with the disease activity.

MATERIAL AND METHODS

Forty two consecutive patients with already established diagnosis of UC hospitalized in the Department of Gastroenterology and Hepatology, Wroclaw Medical University, Poland, were subjected to the study. The group consisted of 32 women and 10 men (mean age 43.05 +/- 10.22 yrs) with an average BMI of 24.35 +/- 3.96 kg/m². Clinical data were gathered at the time of blood and stool sampling. Clinical and endoscopic activity of UC was estimated according to Rachmilewitz’s index. Clinical activity equalled 6.51 +/- 4.13 points, whereas endoscopic activity 5.22 +/- 2.96 points. Six patients suffered from severe UC, 25 mild or moderate disease and 11 were in remission at the time of hospitalization. Erythrocyte sedimentation rate (ESR) ranged from 4 to 120 (mean 22.4/1 h). CRP was elevated (> 6.3 mg/L) in 8 subjects (7.0-84.4, mean 29.1 mg/L). In terms of the disease duration, it ranged from 6 months to 20 years (mean 6.3 +/- 5.67 yrs). Concerning the localization of the gut involvement, UC was limited to the rectum in 21 patients whereas 15 subjects suffered from proctosigmoiditis and 6 from pancolitis. At the time of the study the patients were receiving the following specific treatment: oral 5-aminosalicylate only: 29 subjects, 5-aminosalicylate together with oral prednisone: 10 subjects, oral 5-aminosalicylate and hydrocortisone hemisuccinate intravenously: 3 subjects. All patients with previous history of abdominal surgery related to the IBD course, a stenosis or a fistula revealed on recent endoscopic or radiologic examination were not included in the study. Patients with diabetes mellitus, taking antibiotics and/or antifungal therapy in the last month prior to the hospitalization were excluded from the study.

Informed consent was given by every participant and the study protocol conformed to the Declaration of Helsinki and was approved by the local medical ethics committee at Wroclaw Medical University (ethical committee agreement no. 412).

IL-10 serum concentration testing

IL-10 serum concentration from fasting blood samples was measured with commercial ELISA kit QuantikineHS, Human IL-10 Immunoassay (R&D Systems Inc., catalogue number HS100B, USA) according to the manufacturer’s instructions. Briefly, this method employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-10 was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any IL-10 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-10 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. After an incubation period, an amplifier solution was added to the wells and colour developed in proportion to the amount of IL-10 bound in the initial step. The colour development was stopped and the intensity of the colour was measured. IL-10 serum concentration less than 0.5 pg/mL was regarded as negative.

AMCA detection

The serum level of AMCA from fasting blood samples was measured with commercial ELISA kit Plateia Candida Ab (BioRad, France) according to already described
manufacturer’s instructions [16]. To be brief, microplates coated with cell wall mannan from *C. albicans* VW32 were used in the test. Serum (100 μl) diluted 1/6500 was added to each well and the plates were incubated. After washing, peroxidase-conjugated anti-human immunoglobulins were added, which bound to the anti-mannan antibodies present in the serum. The immunological reaction was revealed by the development of a coloured enzymatic reaction with intensity proportional to the antibody concentration. A standard dilution curve allowed the determination of anti-mannan antibody concentration. The test detects total AMCA (IgG, IgM, IgA). The minimal detectable antibodies level was 2.5 U/mL.

**Mycological stool examination**

Mycological investigation of the stool was performed in all subjects, including the quantitative and qualitative fungal examination. The stool samples (1-2 g) were treated with 0.03% trypsin solution and cultured quantitatively on Sabouraud agar plates supplemented with chloramphenicol. After 48-72 h of incubation at 37 and 28 Celsius degrees the colonies were counted and subcultured on Chromagar Candida (Becton Dickinson, USA) and Rice media. Quantitative mycological investigation was performed according to Müller’s method [17]. Isolates were identified basing on morphological features and biochemical tests (ID-32C, BioMerieux, France). Moulds were identified on the basis of their morphology. The results of at least 10⁶ CFU/g (colony forming units per one gram of stool) were considered as significant fungal colonization (SFC).

**Statistics**

Obtained results were analysed statistically on the basis of Statistica Software 6.0 (Statsoft, Cracow, Poland) with the help of a set of statistical tests (the Student’s *t*-test, the Mann-Whitney *U* test and the Spearman rank correlation coefficient, χ² test and Fisher’s test) for parametric and non-parametric variables. *P*-values less than 0.05 were considered significant.

**RESULTS**

IL-10 serum concentration was measured in 31 out of 42 subjects. In 20 patients (7 in remission, 12 with mild and moderate disease and 1 with severe course of UC) IL-10 serum concentration was below the test sensitivity. In 11 patients (4 in remission, 2 with mild, 3 with moderate disease and 2 with severe course of UC), IL-10 concentrations ranged between 0.78 and 9.43 (mean 3.38 +/- 2.8) pg/mL. Higher serum concentrations of IL-10 were observed only in a few patients with UC with diverse clinical and endoscopic course of the disease. Statistical analysis showed that IL-10 serum concentration correlated neither with clinical (*r* = 0.25; *p* = 0.17) nor with endoscopic (*r* = 0.07; *p* = 0.7) activity (Fig. 1, Fig. 2). No correlation was observed between the concentration of IL-10 and laboratory parameters like serum CRP (*r* = 0.34; *p* = 0.06) or ESR (*r* = 0.31; *p* = 0.08).

AMCA were observed in 8 (19.04%) subjects, all in an active phase of the disease, and their levels ranged from 4.4 to 19.5 (mean 10.72) U/mL. However, no statistically significant difference was found between the serum levels of AMCA in patients with diverse activity of UC (*r* = - 0.28; *p* = 0.12 and *r* = - 0.06; *p* = 0.75, for clinical and endoscopic activity, respectively).

Stool cultures were negative in only 6 patients. In the majority of the subjects (36 out of 42) mycological examination showed the presence of fungi with the predominance of *C. albicans* found in 33 (91.66%) subjects (Tab. 1). Other yeasts
of the genus *Candida* were represented by *C. glabrata*, *C. krusei*, *C. inconspicua*, *C. kefir*, *C. parapsilosis* and *C. ciferri*. Rarely, other species like *Geotrichum spp.* (in 6 patients), *Rhodotorula spp.* (1 patient), *Rhizopus nifer* (1 patient), *Cryptococcus spp.* (1 patient), *Penicillium spp.* (1 patient) were found. However, SFC was revealed in only 3 (8.33%) patients with positive fungal cultures. All subjects with SFC suffered from active UC with clinical activity index ranging from 9 to 12 points. In 2 patients *C. albicans* was cultured, whereas in the third one 2 fungal species were found in the stool sample (*C. albicans* and *Geotrichum spp.*). There were no significant differences between patients during steroid therapy or without steroid therapy as far as AMCA level, fungal colonization as well as the disease duration are concerned. There was also no correlation between IL-10 serum concentration and fungal colonization (r = 0.17; p = 0.36) as well as AMCA serum concentration (r = 0.2; p = 0.28) in the study group.

**DISCUSSION**

In our study we focused on the levels of circulating immunoregulatory cytokine IL-10, which have raised large interest in UC. IL-10 was originally identified as a product of mouse T helper 2 (Th2) clones that suppressed the production of cytokines by T helper 1 (Th1) clones responding to the stimulation by antigen in the presence of monocyte/macrophage antigen-presenting cells [18, 19]. As a down-regulator of the cell-mediated immune response, IL-10 suppresses the production of prostaglandin E2 and numerous pro-inflammatory cytokines, including TNF-α, IL-1, IL-6 and IL-8 [20]. IL-10 also enhances the release of soluble TNF receptors and inhibits the expression of surface ICAM-1 and B7 [21]. Finally, IL-10 has been reported to suppress the synthesis of superoxide anion plus reactive oxygen intermediates (ROI) and either inhibit or facilitate NO synthesis, depending on the time of exposure to activated macrophages [22]. This facilitation is one of many stimulatory functions on monocytes and macrophages that includes up-regulation of both IL-1ra and CD64, and an increase in antibody-dependent cellular cytotoxicity [23]. These results, taken together, suggest that IL-10 can serve as both a macrophage activator and deactivator and exhibit potent anti-inflammatory activities thus exerting a key role in the regulation of mucosal inflammation.

The anti-inflammatory activity of IL-10 was confirmed in an animal model of IBD [24-26]. Different responses to IL-10 administration suggest that the course of IBD in humans is much more diverse than in animals [27]. There are data claiming that an increased serum concentration of IL-10 correlates with the severity of IBD [28]. In contrast to our results, Kucharczik T. et al [29] revealed increased IL-10 concentrations in active UC, whereas the study by Nielsen O. et al [4] showed no difference between UC and healthy subjects. According to our results, IL-10 serum concentration in UC patients does not correlate with the activity of the disease, although it tended towards higher values in some patients with more severe course of UC. Though Mitsuyama K. et al [30] showed increased serum IL-10 concentrations in patients with active UC, in agreement with our study, no statistical association was observed between the concentration of IL-10 and clinical disease activity or severity as well as serum CRP or ESR. There was also no significant difference between patients with or without steroid therapy, which is an observation concordant with our results.

Despite the fact that in our research serum concentration of IL-10 was below the test sensitivity only in one patient with severe course of UC, some authors claim that it happens even in a fulminant UC and the concentrations vary in the course of the disease [31]. Besides, the degree and the frequency of these increases are significantly lower compared to several other immune mediators. Probably reported contradictory results of serum IL-10 concentrations in IBD may depend on subtle differences in the phase of disease [4, 29] as timing of immunomodulatory cytokine measurement is a critical factor detecting the changes in its concentration. Moreover, increased levels of IL-10 may be present in concomitant bacterial infections in severe UC [31].

The presence of fungi and their mannans, which are potent IFN-α inducers, may predispose to disease exacerbations, mucosal lesions or fungal generalized infections. Factors predisposing to candidiasis are often found in UC patients and the diagnosis is challenging due to nonspecific clinical signs and the natural commensal status of these opportunistic pathogens. Previous reports have suggested that the combined serological detection of mannanemia and anti-mannan antibodies may be useful for the diagnosis of systemic candidiasis [32]. However, the presence of *C. albicans*-specific IgM and IgG antibodies in serum samples and the presence of *C. albicans* in stool samples did not exhibit any significant difference between UC patients in active stage and in remission compared with control patients [33], which is in agreement with the results of our study. Anti-mannan antibodies have been shown to be ubiquitous in human sera [34], and in the study of 57 UC patients the appearance of higher AMCA titers entailed a benign course whereas lower AMCA titers were associated with a severe course of the disease [35]. The authors suggest that AMCA may be used as an indicator of the clinical outcome. On the basis of our research, such conclusion cannot be neither proved nor denied, as we did not perform serial measurements of AMCA.

Alterations of the bacterial microflora are regarded as an important factor triggering the disease and may explain the efficacy of antibiotic treatment in active phase of UC. Fungal overgrowth may be the complication of any bacterial imbalance with pathophysiological consequences for mucosal barrier. There is evidence for the deteriorating influence of *Candida* on the healing process of inflamed colon and benefits from probiotics and antifungal treatment in UC [36, 37]. Zwolińska-Weislo M. at al [38] found that SFC (*C. albicans* isolated in 91.7% cultures) was more frequently present in
patients with UC history over 5 years in comparison with shorter disease duration (33.3% and 13.8%, respectively) but the total mean activity indices of inflammation in patients with significant and insignificant fungal colonization of the colon did not demonstrate differences between these groups. However, after 4 weeks a stronger decrease of the UC activity index was observed in the patients with SFC treated with antifungal agents in comparison with the patients not given antifungal therapy. In contrast to the study, we did not show that SFC was more prevalent in UC patients with a disease history longer than 5 years, although one of our subjects with SFC has been suffering from UC for 16 years (the remaining two for 1 and 4 years). However, the number of subjects with SFC was very low in our investigation, so it is difficult to compare the results.

There are interesting studies concerning IL-10 production in relation to C. albicans infection [39, 40]. Tomnetti L. et al [41] showed that the effects of exogenous IL-10 in mice with candidiasis were critically dependent on the dose administered; the highest doses of IL-10 exacerbating the infection. In contrast, at lower doses, IL-10 paradoxically ameliorated the infection. There is also a positive regulatory loop between IL-12 and IL-10 that negatively affects the innate antifungal response, but is required for the induction of optimal adaptive immune response to C. albicans.

It seems that highly sensitive to C. albicans TNF-α and IL-1β release to the GI mucosa rather than IL-10 serum concentration is associated with the intensity of fungal colonization and the fact may be responsible for the lack of correlation between IL-10, AMCA serum titres and SFC in our study. In addition, estimation of the in situ IL-10 concentration by looking for the mRNA expression of IL-10 gene in colon biopsies would be more credible for the analysis of correlation between this anti-inflammatory cytokine and UC activity.

CONCLUSIONS

According to our study, there is no correlation between IL-10 serum concentration in UC patients and the activity of the disease. Therefore, IL-10 serum concentration cannot be a universal marker used in the assessment of the disease severity in UC. Additionally, IL-10 serum concentration does not correlate with the level of AMCA and fungal colonization of the GI tract in UC patients. Moreover, AMCA and SFC are present in the minority of UC patients suggesting that yeasts have minor, if any, influence on the clinical course of the disease.

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