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Different Patterns of Serum Interleukin 10 Response to Treatment with Anti-Tumor Necrosis Factor α Antibody (Infliximab) in Crohn´s Disease

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Summary

Administration of anti-tumor necrosis factor antibody (anti-TNF, infliximab) down-regulates T helper 1 (Th 1) cytokines production in intestinal mucosa of patients with Crohn´s disease (CD). Interleukin 10 (IL-10) is thought to be involved in CD pathogenesis through regulation of the Th 1 response. The aim of this study was to determine the IL-10 response in CD patients treated with anti-TNF. Fourteen patients with active CD received 5 mg/kg of infliximab; clinical activity assessed by Crohn´s Disease Activity Index (CDAI), α1-acid glycoprotein and serum IL-10 were determined before and after treatment, in month 0, 1 and 5. In the group with a good clinical response, IL-10 levels diminished significantly in month 1 ($p<0.05$) and remained decreased in month 5. The group with a lower response showed a significant increase in IL-10 levels in month 1 ($p<0.05$). α1-acid glycoprotein levels obtained before treatment were significantly elevated in the group with a good clinical response ($p<0.05$) and a significant decrease in month 1 was observed in this group ($p<0.05$). These observations suggest that a pattern of IL-10 response might be related to the clinical response to anti-TNF treatment in CD.

Key words

Crohn´s disease • Anti-tumor necrosis factor therapy • Interleukin 10 • α1-acid glycoprotein

Introduction

The etiology of Crohn´s disease (CD) is still not fully elucidated and therefore the causal therapeutic agent for this disorder is lacking. However, recent years have brought new insights into the CD pathogenetic pathways with stress on immune mechanisms and genetics. Increased production of proinflammatory cytokines was observed in CD (Pullman *et al.* 1992, Mullin *et al.* 1992, Breese *et al.* 1993) and these molecules seem to play an important role in triggering and further maintaining mucosal inflammation. Therefore, novel therapeutic possibilities appeared, aiming at the suppression of key T helper 1 (Th 1) cytokine, tumor necrosis factor (TNF) α. These anti-TNF strategies comprise agents inhibiting TNF production or secretion, monoclonal antibody against TNF-α and TNF receptor fusion proteins (Baert and Rutgeerts 1999). Infliximab is a monoclonal,

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chimeric antibody against TNF-α molecule. Although its precise placement in the therapeutic strategy of CD is to be evaluated, the results of the use of anti-TNF treatment in active CD, resistant to conventional therapy (5-aminosalicylic acid-containing drugs, corticosteroids, azathioprine) are promising (van Dullemen *et al.* 1995, McCabe *et al.* 1996, Targan *et al.* 1997, D´Haens *et al.* 1999).

Moreover, immunoregulatory cytokines are also involved in the pathogenesis of CD. Increased serum levels of interleukin 10 (IL-10) were observed in patients with active CD and ulcerative colitis (UC), suggesting that IL-10 acts as a naturally occurring damper in the acute inflammatory process of inflammatory bowel disease (Kucharzik *et al.* 1995).

Thus, the administration of anti-TNF antibody might be associated with changes in both proinflammatory and regulatory parts of the immune system. In an attempt to assess the pattern of immunoregulatory cytokine response in CD patients treated with anti-TNF antibody, serum levels of IL-10 were measured together with clinical and laboratory parameters of disease activity.

Methods

The study was conceived as a prospective clinical trial. Its realization was approved by the Institutional Ethics Board and informed consent was obtained from each patient.

Fourteen patients (9 women and 5 men), with active, moderate to severe Crohn´s disease were included. Mean age of patients was 36 years, ranging from 21 to 42 years and mean duration of the disease was 10.7 (range 1 to 24 years) years. Thirteen patients were receiving 5-aminosalicylic acid-containing drugs and 3 patients were treated by corticosteroids in the last year before being involved in the study. Patients received 5 mg per kg of anti-TNF antibody (infliximab) in intravenous infusion. One patient did not receive any concomitant medication, other 13 patients were receiving 5-aminosalicylic acid-containing drugs and corticosteroid medication continuously during the whole follow-up.

Clinical activity (in 14 patients), serum IL-10 (in 14 patients), serum α1-acid glycoprotein (in 12 patients), basic hematological and biochemical parameters (blood count, prothrombin time, renal and hepatic functions) were assessed. All parameters were obtained before treatment (Month 0) and in Months 1 and 5 after treatment.

Clinical activity was assessed by Crohn´s disease activity index (CDAI) (Best *et al.* 1979) that represents a composite measure of frequency of loose bowel movements, severity of abdominal pain, general well-being, extraintestinal manifestations, presence of abdominal mass, use of antidiarrhoeal drugs, hematocrit and body weight.

Serum α1-acid glycoprotein levels were obtained by the immunodiffusion method (Laurell 1966) using a monospecific antiserum (A_1Oroso, USOL Prague). Serum IL-10 was measured by a commercially available kit Quantikine® (R&D Systems) that employs the quantitative sandwich enzyme immunoassay technique with a microplate coated with murine monoclonal antibody against IL-10. The minimum detectable dose of IL-10 with this kit is typically less than 3.9 pg/ml.

Non-parametric statistical analysis was applied. Median and ranges are given. Data were tested by Wilcoxon´s test. A significance limit of 0.05 was used.

Results

The treatment was well tolerated, no side effects related to treatment with anti-TNF were observed during the whole follow-up. Basic hematological and biochemical parameters remained unchanged and were within the range of normal physiological values, except for two patients with mild anemia attributed to iron deficiency.

Clinical improvement was observed in 12 patients with a decrease in median CDAI from 228 (163-294) before treatment to 98.5 (56-160) in Month 1; two patients did not respond.

According to the clinical response in Month 1, patients were divided into two groups: group 1 (7 patients) with a decrease in CDAI of 50 % and more; in this group the median of CDAI before treatment was 240 (169-294) diminishing in Month 1 to 81 (56-125); and the group 2 (7 patients) with a drop of CDAI less than 50 %; the median of CDAI in this group was 265 (163-300) before treatment and it decreased to 145 (114-294) after 1 month. During the further clinical follow-up, patients in the group 1 remained stable with CDAI of 82 (28-216) in Month 5, while the clinical activity in the group 2 rose to 203 (108-318) in Month 5 that did not differ significantly from the clinical activity before treatment (Table 1). All three patients receiving

concomitant corticosteroid medication belonged to the group 1.

Measurement of α1-acid glycoprotein, a laboratory marker of inflammatory activity, showed significantly higher levels in group 1 before treatment ($p<0.05$). A significant decrease in Month 1 was observed in group 1 ($p<0.05$) with a further increase above the values before treatment in Month 5 (Table 1), whilst there was not a clear-cut pattern of α1-acid glycoprotein response to treatment in group 2.

IL-10 levels before treatment ranged from 3.62 pg/ml to 6.08 pg/ml with a median of 4.44 pg/ml in 13 patients; in one case the IL-10 levels were elevated up to 22.72 pg/ml. During the further follow-up, there was a significant decrease in IL-10 levels in the group 1 ($p<0.05$) in Month 1 and IL-10 levels remained decreased compared to values before treatment in Month 5 (Table 1, Fig. 1). On the other hand, in group 2 a significant increase in IL-10 levels ($p<0.05$) was observed in Month 1, without significant changes in Month 5 (Table 1, Fig. 1).

Table 1. CDAI, interleukin 10 and α-1-acid glycoprotein (expressed in medians and ranges) in two groups of patients.

	CDAI	Interleukin 10 (pg/ml)	α-1-acid glycoprotein (mg/l)
Group 1 (No. of patients)	7	7	6
Month 0	240 (169-294)	5.29 (4.44-22.72)	1120 (690-2040)
Month 1	81 (56-125) *	4.81 (3.45-18.57) *	780 (530-1360) *
Month 5	82 (28-216) *	3.98 (3.36-20.29) *	1375 (750-1800) *
Group 2 (No. of patients)	7	7	6
Month 0	265 (163-300)	3.99 (3.62-4.62)	793 (710-1400) #
Month 1	145 (114-294)	4.07 (3.80-5.49) *	810 (700-1060)
Month 5	203 (108-318)	4.16 (3.71-5.29)	905 (700-1620)

**p<0.05 when compared with Month 0; # p<0.05 when compared with the group 1.*

Discussion

In the present study we observed that different clinical responses to the treatment with anti-TNF antibody were associated with specific patterns of changes in IL-10 levels. With respect to the different clinical responses (assessed by CDAI), the levels of IL-10 were as follows: 1) in the group of patients with a good clinical response IL-10 levels diminished significantly; 2) in the group with a lower response to the treatment a significant increase of IL-10 was observed after one month and no significant changes were noticed during the further follow-up.

IL-10 plays an important role in the regulation of T cell response. It suppresses lipopolysaccharide-induced production of IL-1, IL-6 and TNF-α by macrophages (Fiorentino *et al.* 1991) and the increased production of these Th 1 cytokines is considered to play a pivotal role in CD pathogenesis. Increased levels of IL-10 were observed in patients with active CD (Kucharzik *et al.* 1995) suggesting that this cytokine may be important for the down-regulation of the Th 1 response and its production could be related to the production of proinflammatory cytokines. Anti-TNF therapy reduced the number of intercellular adhesion molecule 1 and lymphocyte function-associated antigen 1 expressing and IL-4- and TNF-positive lamina propria mononuclear cells (Baert *et al.* 1999) and the number of lamina propria mononuclear cells producing TNF and IFN-gamma when activated with CD2/CD28 (Plevy *et al.* 1997). Thus, the decrease in IL-10 levels in the group of patients with a good clinical response to anti-TNF therapy might reflect sufficient suppression of the Th 1 response by the treatment and *vice versa*. Moreover, the pattern of the IL-10 response might play a role in determining the response to anti-TNF therapy. Recently, an observation of antineutrophil cytoplasmic antibody (ANCA) and lymphotoxin α (LTA) haplotype relationship to clinical response to anti-TNF treatment in CD was reported, suggesting that speckled ANCA may identify a CD

subgroup with a better response to anti-TNF and that perinuclear ANCA and homozygosity for the LTA 1-1-1-1 may identify subgroups with a poorer response (Taylor *et al.* 2001). This may suggest that there are subgroups differing in the response to the anti-TNF treatment because of their specific cytokine patterns in which IL-10 might be involved.

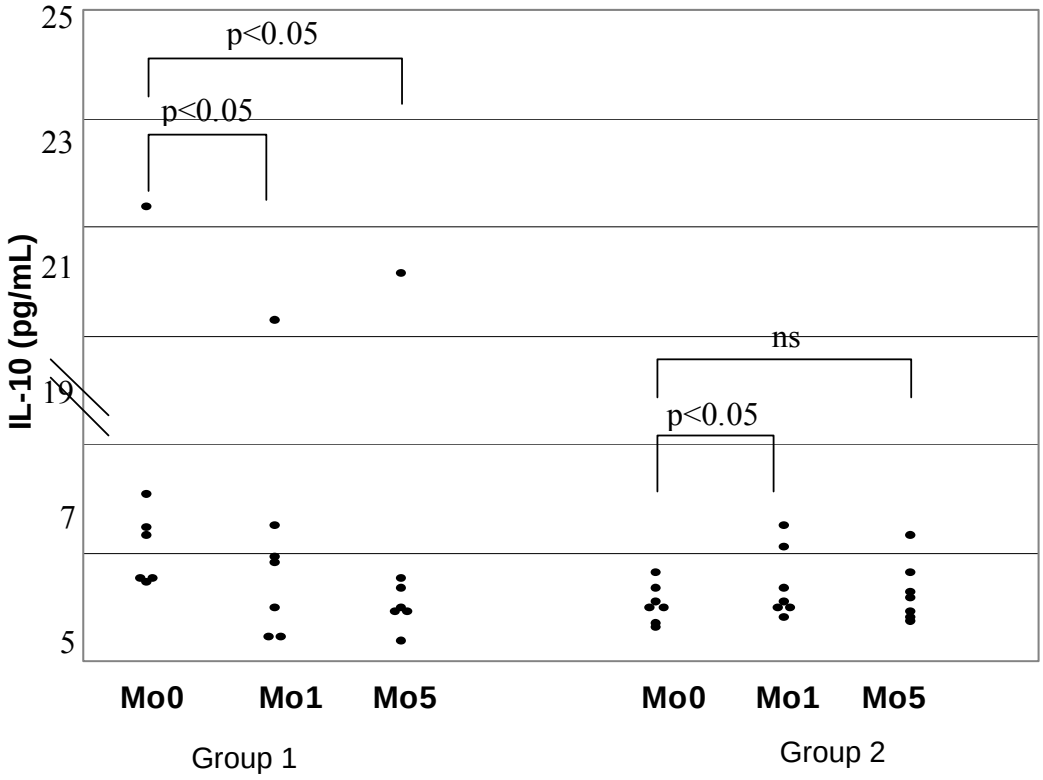

Fig.1. *Changes in IL-10levels in group 1 and 2*

α-1-Acid glycoprotein, a reliable biological marker of inflammatory activity in CD (Lubega and Davies 1990) reflects the clinical activity in CD patients treated with anti-TNF (Kupčová *et al.* 2001). In the present study, the group with a good clinical response showed significantly diminished α-1-acid glycoprotein levels whilst in the group with a poorer response there was no clear-cut pattern of changes in α1-acid glycoprotein levels. Interestingly, the two groups differed in α-1-acid glycoprotein levels before treatment, with significantly higher values obtained in the group with a good clinical response.

α-1-acid glycoprotein is one of the major acute phase proteins, its serum concentration increases in response to systemic tissue injury, inflammation or infection, and these changes in serum protein concentrations have been correlated with increases in hepatic synthesis (Fournier *et al.* 2000). Increased expression of α-1-acid glycoprotein mRNA was reported in cultured normal rat hepatocytes when stimulated by dexamethasone associated with various Th 1 cytokines (Barraud *et al.* 1996). As mentioned above, the increased Th 1 cytokine production is considered to play an important role in the pathogenesis of CD. Moreover, it has been suggested that the effect of anti-TNF therapy may depend on the pattern of Th 1 activation (driven by high levels of TNF or other cytokines) (Taylor *et al.* 2001). Thus, one can speculate that the elevated α1-acid glycoprotein levels might be related to a more pronounced Th1 activation, expecting then a better clinical response to anti-TNF treatment in these patients. However, during the further follow-up of the group 1, an increase of α1-acid glycoprotein above the levels before treatment was observed in Month 5, together with a persistent good clinical condition. It seems that this laboratory finding could precede a clinical relapse (Brignola *et al.* 1986, Wright *et al.* 1987, Louis *et al.* 1997) and therefore a close follow-up of these patients is

necessary with consideration of further therapeutic intervention.

Limitations of the study presented here are a small size of the cohort and a limited number of parameters evaluating the Th 1 and Th 2 response. Further investigations, with larger number of patients and complete assessment of the immune status are needed to confirm the hypothesis presented here about the relationship of the IL-10 specific pattern to the clinical response to anti-TNF treatment.

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