

# Possible defect of regulatory T cells in patients with ulcerative colitis

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## Abstract

**Background:** The dysfunction of regulatory T cells (Tregs) has been implied in the development of several autoimmune and inflammatory diseases. Our project aimed to investigate the frequency of peripheral Tregs in patients with active ulcerative colitis (UC), a chronic relapsing and remitting inflammatory gastrointestinal tract condition.

**Material and methods:** A group of 40 adults with active UC was enrolled. The disease had been diagnosed and confirmed by endoscopic means. Only patients treated with conventional therapies were recruited to the study. A cytometric analysis was performed to evaluate the levels of peripheral Tregs and interleukin 10 (IL-10) in the investigated patients.

**Results:** A statistical analysis showed a significantly lower percentage of Tregs in the UC patients compared with the control patients ( $p < 0.05$ ), while no significant difference in the mean levels of serum IL-10 was observed ( $p > 0.05$ ).

**Discussion:** The lower percentage of Tregs observed in the UC patients might result from a numerical and/or functional defect of these cells. Normal, but not elevated, levels of IL-10 in the serum of UC patients might also suggest the numerical defect of Tregs. Further research is needed to evaluate the potential of Treg adoptive transfer method for UC treatment.

**Key words:** regulatory T cells, ulcerative colitis, inflammatory bowel disease.

(*Centr Eur J Immunol* 2011; 36 (4): 254-255)

## Introduction

The main role of forkhead box P3 (FOXP3) + regulatory T (Treg) cells is to maintain immune tolerance and prevent inflammatory diseases [1]. The impaired function and/or homeostasis of these cells has been implied in the development of several autoimmune and inflammatory diseases, including rheumatoid arthritis, lupus erythematosus, multiple sclerosis and type 1 diabetes [2]. A growing evidence suggests that the defect within Treg compartment might also be an underlying cause of an inflammatory bowel disease (IBD), a chronic relapsing and remitting inflammatory gastrointestinal tract condition [3]. The disease manifests as two distinct but often overlapping clinical forms – ulcerative colitis (UC), which pathology is restricted to the colonic mucosa, and Crohn's disease (CD), which can affect any part of the gastrointestinal tract [4]. Our project aimed to investigate the levels of peripheral CD4+CD25+FOXP3+ Tregs in patients with active moderate-to-severe UC.

## Material and methods

The project was approved by the local Ethics Committee. A group of 40 adults with active moderate-to-severe UC comprising 27 females (67.5%) and 13 males (32.5%) of a mean age of  $34.2 \pm 13.1$  years was enrolled. The disease had been diagnosed and confirmed by endoscopic means. Only patients with CRP level  $\geq 10$  mg/ml were eligible. Only patients treated with conventional therapies, including 5-aminosalicylates, prednisolone, budesonide, azathioprine, mercaptopurine, methotrexate and antibiotics, were recruited to the study. The control group was composed of 15 healthy volunteers, 9 females (60%) and 6 males (40%) of a mean age of  $31.6 \pm 9.2$  years that had not been subject to any immunomodulatory therapies. A cytometric analysis was performed to evaluate the levels of CD4+CD25+FOXP3+ regulatory T cells (BioLegend, USA) in peripheral blood of the investigated patients. Also, serum samples were cytometrically tested for con-

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centrations of interleukin 10 (IL-10) (BD Biosciences Pharmingen, USA). The data were analyzed by Mann-Whitney U, a Wilcoxon signed rank test, and  $\chi^2$  test using SPSS version 15. Values were considered significant when  $p < 0.05$ .

## Results

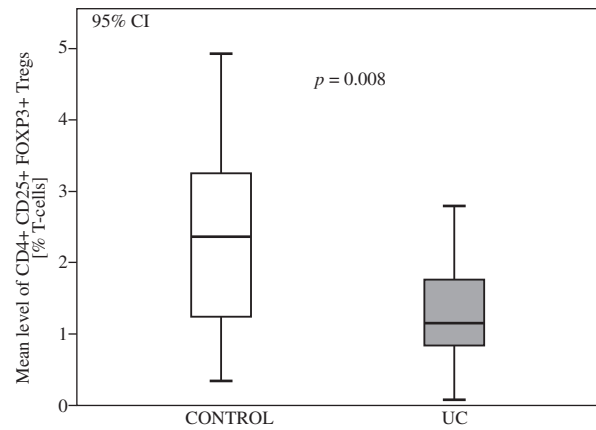
A statistical analysis showed a significant difference in the levels of peripheral CD4+CD25+FOXP3+ regulatory T cells among the investigated groups. A significantly lower percentage of Tregs was found in the active moderate-to-severe UC group compared with the control group ( $1.32 \pm 0.7$  vs.  $2.36 \pm 1.4$ ;  $p < 0.05$ ) (Fig. 1). No significant difference in the mean levels of serum IL-10 was observed among the investigated groups ( $p > 0.05$ ).

## Discussion

Our analysis showed a lower percentage of CD4+CD25+FOXP3+ Tregs in peripheral blood of the UC patients compared with the control group, which is consistent with some reports [3, 5]. The lower percentage of Tregs might result from the numerical and/or functional defect of these cells, which has been implicated in the development of several autoimmune and inflammatory diseases [1]. It is possible that the generation and function of these cells are inhibited by a chronic overproduction of IL-6 and IL-17 found in UC patients [6]. Active blocking by Th17 cells, which stay in a dynamic balance with Treg compartment, is another possibility [7]. Current data demonstrate that active UC is characterized by a numerical defect of regulatory T cell subpopulation rather than by a compromised suppressor function of these cells. In spite of their presence in the inflamed GI tract of CD patients, their number is probably insufficient to control the ongoing inflammation [3]. It is worth noting that the low percentage of peripheral Tregs found in the active moderate-to-severe UC patients is accompanied by normal serum levels of IL-10. Interleukin 10 is a potent anti-inflammatory and immunoregulatory cytokine produced mainly by Tregs and its presence appears to be required for suppression of colitis in mouse models [8, 9]. Normal, but not elevated, levels of IL-10 in the serum of UC patients might also suggest the numerical defect of Tregs. Concluding, Tregs have a potent anti-inflammatory capacity in animal models of colitis. It seems that patients with active moderate-to-severe UC also exhibit a numerical dysfunction within Treg compartment. Therefore, further research is needed to evaluate the potential of Treg adoptive transfer method for UC treatment.

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**Fig. 1.** Mean level of CD4+CD25+FOXP3+ regulatory T cells in peripheral blood of healthy volunteers (CONTROL) and patients with active ulcerative colitis (UC) (values shown as % T cells)

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