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THE EFFECT OF EXPERIMENTAL ILEITIS IN RATS ON EXPRESSION OF THE TRANSCRIPTION FACTOR FOXP3

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Crohn's disease and ulcerative colitis are the main clinical phenotypes of inflammatory bowel disease (IBD). Both forms of IBD can increase the incidence of gastrointestinal and colon cancers, and both ones are associated with significant morbidity and mortality worldwide. In addition, they can begin early in life and persist for long periods [1, c. 575]. The pathogenesis of IBD is complex and multifactorial. T helper cells are components of the adaptive immune response whereas Toll-like receptors, NOD-like-receptors, RIG-I-like receptors are involved in maintaining mucosal as well as commensal homeostasis. Foxp3 is a member of the family of transcription factors and plays a critical role in the development regulatory T cells (Tregs). Tregs important for controlling inflammation in the intestine in patients with IBD [2, c. 648; 3, c. 2849]. **The aim of this research** was to investigate the effect of acute ileitis on expression intensity of the transcription factor Foxp3 with lymphocytes of small intestine. **Materials and methods.** Male Wistar rats weighing 200–250 g were housed in standard wire-mesh bottom cages at constant temperature of 25°C and 12/12 h light/dark cycles. For induction of an acute ileitis, rats received one subcutaneous dose of indomethacin (15 mg/kg). Structure of population of $Foxp3^+$ has been studied by the analysis of serial histological sections using the method of indirect immunofluorescence with monoclonal antibodies. After deparaffinization sections were rehydrated in graded ethanol (100% twice, 90% once, 80% once, and 70% once, each for 5 min), washed in PBS (twice, 5 min each). After rinsing in 0.1 M PBS, the sections were incubated for 1 h at room temperature with anti-Foxp3 FITC-conjugated antibodies. While protected from direct light exposure, samples were washed three times in PBS and mounted. Fluorescent images were obtained with a fluorescence microscope PrimoStar (ZEISS, Germany) with a computer-assisted video system AxioCam 5c (ZEISS, Germany). Fluorescent signal

intensity was quantified using ImageJ software (NIH Image version 1.46). The levels of Foxp3 expression were expressed as the relative mean fluorescence intensity in arbitrary units (AU). For the indomethacin ileitis model, the gross inflammatory scores were assessed macroscopically according to a recently described scale [4, c. 1250]. Gross inflammatory scores (0–7) were assigned using each ileum was assigned a score on this scale ranging from 0 (normal) to 7 (severe damage). All statistical analyses were performed using EXCEL MS Office 2010 (Microsoft Corp., USA), STATISTICA 6.0 (Stat-Soft, 2001) software. Results are expressed as mean values ± SEM. Differences were considered statistically significant if the p value was <0.05. **Results.** Indomethacin produced numerous palpable nodules on the serosal side of the ileum as well as dilated, adherent, and hyperemic areas in the mid part of the small intestine. Adhesions between adjacent intestinal loops were observed, as well as mesentery and epididymal adipose fat pads and mesentery bleeding. When the intestinal lumen was opened longitudinally, it was found that indomethacin had invariably produced multiple deep longitudinal ulcers with many round lesions. These ulcers involved the entire circumference of the bowel wall throughout the length of the ileum. The mean gross inflammation index was increased to 5.1 ($p<0.05$), compared with control values for rats receiving 5% sodium bicarbonate (1.2, $p<0.05$). It has been established that development of acute ileitis was accompanied with 29-32% ($p>0,05$) decrease in quantity of Foxp3$^+$-cells and it led to the decrease concentration of the transcription factors Foxp3 immunopositive cells. **Conclusion.** Development of acute ileitis was accompanied with the decrease in quantity of Foxp3$^+$ cells and it influenced concentration of the transcription factors in immunopositive cells.

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