

Abstract

Introduction: Celiac disease is identified as the loss of oral tolerance to gluten, it is an organ-specific autoimmune disease in which both, adaptive and innate immunity participate. Monocytes are important part of immune system; they have many functions and express very diverse membrane receptors including Toll-like receptors (TLRs). TLRs are involved in the innate immune response, specifically TLR2 and TLR4 are crucial for recognition of bacterial components and TLR7 recognizes virus's ssRNA. Monocytes also produce prolactin (PRL), which acts as a cytokine that modulates immune responses. To clarify the role of innate immunity and circulating monocytes in pathogenesis of celiac disease, we focused on changes in expression of selected Toll-like receptors (TLR2, TLR4, TLR7), prolactin, some pro- a anti-inflammatory cytokines (TNF- α , IL-6, IL-12, IL-10). We monitored the influence of the SNP – 1149 G/T on the expression of PRL mRNA. Another objective of this work was the introduction and optimization of *in vitro* methods for cultivation and stimulation of peripheral monocytes.

Material and Methods: This pilot study includes 21 patients with celiac disease and 40 healthy controls. For determination of mRNA levels of the studied genes we isolated RNA from monocytes that were isolated by immunomagnetic separation. Quantitative testing was performed using Real Time PCR with *PGKI* as endogenous control. To detect *PRL* - 1149 G/T SNP we used PCR-RFLP method with the *XapI*.enzyme

Results: The levels of TLR2, TLR4 and TLR7 mRNA in peripheral blood monocytes were significantly increased compared to levels in controls, specifically TLR2 mRNA $52,4 \times$ ($P < 0,0001$), TLR4 mRNA $7,8 \times$ ($P < 0,0001$), TLR7 $20,3 \times$ ($P < 0,0001$). Expression of PRL mRNA was not statistically significantly increased in patients with celiac disease ($P = 0,9$). There was no association between *PRL*-1149 and the PRL mRNA expression ($P = 0,1483$). Significantly decreased expression of TNF, IL-6 a IL-10 mRNA in patients compared to controls was detected: TNF $103,2 \times$ ($P < 0,01$), IL-6 $10,9 \times$ ($P < 0,0001$) and IL-10 $16,25 \times$ ($P < 0,0001$). We observed $2,97 \times$ significantly higher expression of IL-12 mRNA in controls in comparison to patients with gluten free diet.

Conclusion: These results suggest that the immune dysbalance in the form of celiac disease apparently leads to increased expression of studied Toll-like receptors (TLR2, TLR4, TLR7 mRNA) and to reduced of expression of anti-inflammatory IL-10. Increased expression of pro-inflammatory cytokines observed in controls may be caused by late

processing of buffy coats from donors. It appears that the SNP -1149 does not affect the expression of PRL mRNA.

Key words: celiac disease, monocytes, prolactin, Toll-like receptors, cytokines