Abstract

Introduction: Celiac disease is an organ-specific autoimmune disease that results in loss of oral tolerance of gluten in genetically predisposed individuals. Increased levels of inflammatory cytokines (eg IFN-γ, TNF-α, IL-6) in the small intestine and peripheral blood have been observed in patients with celiac disease. These cytokines have the ability to increase the expression of interferon regulatory factor 1 (IRF1), which is a potential player in the pathogenesis of celiac disease. IRF1 plays a role in apoptosis, differentiation and anti-inflammatory response of various immune cells. Monocytes are an important component of the immune system. A wide variety of their functions allow them to exertmembrane receptors, whose expression may cause, among other things, by IRF1. To clarify the effects of IRF1 on the pathogenesis of celiac disease we have focused on circulating monocytes in this pilot study and (i) detection changes in the monocytic expression of IRF1 mRNA in inflammatory environments in patients with celiac disease (recent, rCD and adherent one year and longer gluten-free diet, CD-GFD), and ii) subsequently to reveal the effect of IRF1 on monocytes by apoptosis markers (CD95) and anti-inflammatory responses markers (CD163 and IL-10).

Material and methods: The study includes 15 patients with celiac disease (5 rCD and 10 CD-GFD) and 10 healthy donors as controls. For the determination the mRNA levels of the studied factor and cytokines (IRF1, CD95, CD163, IL-10), we isolated RNA from the monocytes, which was obtained using FicollPaque density gradient separation; quantitative RNA testing was performed by the method of QPCR with *PGK1* as endogenous control.

Results: The levels of IRF1 mRNA in monocytes from recent patients, gluten-free patients and healthy donors were significantly increased after stimulation with different cytokine combinations (IFN-γ, IFN-γ + TNF-α and IFN-γ + TNF-α + IL-6). In recentních patients the expression increased by six times ($p < 0.05$), in patients on a gluten-free diet 7.7 times ($p < 0.0001$) and 7 times in healthy donors ($p < 0.0001$). Expression of target markers CD95, CD163 and IL-10 in IFN-γ and TNF-α stimulated monocytes from CD-GFD patients showed trends in increased expression of apoptotic marker CD95 mRNA and decreased expression of anti-inflammatory marker IL-10 mRNA (p = NS); reduction of CD163 mRNA expression in stimulated monocytes compared to cytokine-free monocytes was significant ($p = 0.008$). In monocytes from HC, were observed statistically insignificant trends of increased expression of apoptotic marker CD95 mRNA and decreased expression of anti-inflammatory markers CD163 and IL-10 (p = NS). However, expression of IRF1 mRNA did not correlate with any

of the selected markers, nor in monocytes of healthy donors, nor in patients on gluten-free diet (p = NS).

Conclusion: The results suggest that the inflammatory environment represented by cytokines IFN-γ, TNF-α, IL-6, which are present in increased levels in the serum of recent patients, leads to increased expression of IRF1 mRNA in monocytes, which can subsequently trigger apoptosis monocytes by increasing CD95 expression mRNA and decrease the expression of anti-inflammatory markers CD163 mRNA and IL-10 mRNA. This may indicate a reduction in the immune response of monocytes in these patients.

Key words: celiac disease, monocytes, cytokines, interferon regulatory factor 1 (IRF1), apoptosis, inflammation.