Abstract

Introduction: Bone is a highly active tissue throughout life and is a subject to constant remodelling. Main cells responsible for continuous resorption and *de novo* synthesis of bone matrix are osteoclast, osteoblasts and osteocytes. Osteoclasts are the only known type of cells able to resorb bone. These cells are formed by fusion of precursor cells in bone marrow or peripheral blood in a process called osteoclastogenesis. Formation of osteoclasts may be of importance concerning chronic inflammatory diseases that are linked with higher risk of developing osteoporosis during lifespan. Celiac disease is one of those diseases, which is characterized by destruction of intestinal mucosa after ingestion of gluten by susceptible individuals followed by induction of chronic inflammation. In this work, we focused on the potential role of osteoclastogenesis in the development of osteoporosis in patients with celiac disease and we studied roles of selected inflammatory agents (TNF-α, IL-6, IFN-γ a cfDNA) with supposed or hypothesised effects on osteoclastogenesis.

Material & Methods: We obtained plasma and serum samples from newly diagnosed patients with celiac disease, patients on gluten free diet and healthy controls and analysed concentrations of cfDNA and inflammatory cytokines TNF-α, IL-6 and IFN-γ in those samples. Sera of these patients and controls were then used for subsequent *in vitro* cultivations. To verify effects of cfDNA, same sera were also used after DNase treatment. To verify effects of celiac sera and inflammatory factors on osteoclastogenesis, two-week cultivations of monocytes separated from peripheral blood of healthy donors were performed in presence of these factors. Osteoclastogenesis was then evaluated by count of osteoclasts using optical microscope and by qPCR quantitation of gene expression of typical osteoclast markers ACP5, CTSK and CALCR.

Results: No differences in concentrations of all studied inflammatory factors were found between groups of celiac patients and healthy donors. In presence of serum of newly diagnosed patients, about 50 % more osteoclasts in comparison to healthy serum have formed on average. IL-6 directly inhibited osteoclastogenesis in our experiments, while TNF-α showed a trend towards positive stimulation of osteoclastogenesis. In case of IFN-γ, we found both stimulatory and inhibitory effects that seem to vary depending on an individual healthy

donor of monocytes. In our pilot experiments with cfDNA, we observed trend towards a several fold decrease of osteoclastogenesis in presence of serum treated with DNase.

Conclusion: Our results suggest there are one or more factors in sera of newly diagnosed patients with celiac disease that may not be directly linked to inflammation, but are responsible for significant increase of osteoclastogenesis in peripheral precursors. This effect may play an important role in higher risk of osteoporosis in celiac disease patients. Regarding inflammatory cytokines, IFN-γ might play an important role in modulation of effects of other cytokines in osteoclastogenesis and its effects seem to be specific for monocytes from each particular healthy donor. The results from cfDNA experiments suggest its potentially significant role in osteoclastogenesis and should be further evaluated using other models and different methods in the future.

Key words: osteoclast, osteoclastogenesis, osteoporosis, inflammation, inflammatory diseases, celiac disease, TNF-α, IL-6, IFN-γ, cfDNA