Abstract

Introduction: Extracellular DNA (ecDNA) is a common component of blood plasma. Increased levels of ecDNA in plasma can be found in some autoimmune diseases like systemic lupus erythematosus (SLE), rheumatoid arthritis or celiac disease which are associated with inflammatory processes. These diseases are also associated with an increased risk of osteoporosis. Bone is a dynamic structure undergoing constant modelling caused by osteoblasts, osteocytes and osteoclasts. Shifting their equilibrium can lead to pathological conditions such as osteoporosis. In this thesis we focused on elucidating whether ecDNA, an inflammatory agent with proven immunoregulatory effects can alter differentiation potential of monocytes and alternatively lead to osteoclastogenesis via TLR9.

Material and methods: We obtained monocytes from peripheral blood of healthy donors and cultivated them with four types of ODNs control (CO), stimulatory (ST), inhibitory (INH, telomeric (TLM) with phosphodiester (-pO) or phosphorothioate (-pS) backbone for two weeks to establish their effect on differentiation potential of monocytes into osteoclasts. Osteoclastogenesis was evaluated by number of yielded osteoclasts observed on a light microscope. To establish the effect of ODNs on osteoclast activity samples were analysed by qPCR for expression of *ACP5, CTSK, TLR9* and *NFATc1*.

Results: No significant differences were found in experiments that tested -pO ODNs when compared to their respective blanks. Out of three control ODNs tested, only CO-ODN-2395 (10 nM) was proven neutral and appropriate as a control ODN for further experiments. ST-ODN-2006 (0.1 nM) proved to stimulate osteoclastogenesis. Both INH-ODN-4347 and TLM-ODN (TTAGGG)4 did not inhibit osteoclastogenesis when tested alone. Combination of INH-ODN (100 nM) + ST-ODN (0.1 nM) exhibited significant difference when compared to ST-ODN (0.1 nM). Combined effect of TLM-ODN (10 nM) + ST-ODN (0.1 nM) did not significantly differ from the effect of ST-ODN (0.1 nM). Changes in mRNA expression did not show significant differences in any gene when the cells were cultivated with various ODNs, their combinations or when the cells were harvested at different times of cultivation.

Conclusion: Our results suggest that -pS ODNs are appropriate for long-term cultivations. The CO-ODN-2395 (10 nM) seems to be a suitable control in cultivations of monocytes. ST-ODN-2006 (0.1 nM) significantly stimulates osteoclastogenesis. Lone INH-ODN and TLM-ODN do not inhibit osteoclastogenesis when added into the culture. When ST-ODN-2006 (0.1 nM) is combined with INH-ODN-4347 (100 nM) the osteoclastogenesis is significantly inhibited. TLM-ODN (10 nM) + ST-ODN (0.1 nM) do not significantly inhibit osteoclastogenesis, but more robust data is needed to verify this effect. Though ST-ODN produces more osteoclasts, their activity was not increased; nevertheless, more experiments are needed to verify this effect. *TLR9* might possibly not be involved in alternatively activating differentiation of monocytes into osteoclasts, but further tests are required to prove its role in osteoclastogenesis.

Key words: oligodeoxynucleotide, ODN, extracellular DNA, ecDNA, inflammation, osteoclastogenesis, monocyte, osteoclast, TLR9, NF-κB