Abstract

Introduction: Diabetes mellitus is a polygenic disease and its development is influenced to some extent by environmental factors as well. Innate immunity triggers nonspecifically first defense reactions after penetration of the pathogen into the body, while overstimulation components of innate immunity may give rise to autoimmune diseases, including diabetes type 1. The components of innate immunity are, among others, Toll-like receptors (TLRs) belonging to a group of the structures recognizing preserved molecular structures characteristic of pathogens. Toll-like receptors are abundantly expressed by monocytes which produce prolactin (PRL) having an immunostimulatory function. To clarify the role of innate immunity in the pathogenesis of diabetes, we focused on the expression of mRNA and protein expression of TLR2 and TLR4. The expression of PRL was studied only at the level of mRNA. Monocytes were separated by flow cytometry into classical (CD14++) and nonclassical (CD14+). We monitored their percentages and the degree of expression of CD14 antigen on their surface.The operational objective of this dissertation was to optimize the stimulation of monocytes for the planned study of the function of non-pituitary prolactin in vitro and determine the appropriateness of the use of healthy donors´ buffy coats as healthy controls.

Material and Methods: In the study there were included 30 patients with autoimmune diabetes (AD), 16 with diabetes type 2 (T2D), 25 non-diabetic patients (nonDM), 25 buffy coats and 24 healthy controls which were analyzed by Real Time PCR with PGK1 as endogenous control to monitor mRNA expression of PRL, TLR2 and TLR4. For the detection of surface proteins 19 AD, 8 T2D, 6 nonDM patients and 24 healthy controls were processed. Buffy Coats mentioned above were used only for the study comparing mRNA expression of PRL , TLR2 and TLR4 buffy coats and healthy controls. For optimization experiments results obtained by processing whole blood from five healthy individuals were presented.

Results: Patients with AD showed increased mRNA expression of TLR2 compared with healthy controls, as for the protein this phenomenon was not observed. TLR4 mRNA expression was not increased both in AD patients compared to controls and even at the level of protein, which was even lower compared to controls. There was no increase in expression mRNA of PRL in patients with AD compared to healthy controls. Patients with AD in comparison with healthy controls have fewer CD14++ and CD14+ cells, but on their surface more CD14 molecules were detected than in healthy controls. Expression of all studied

markers differed in a group of buffy coats of healthy blood donors and healthy individuals collected in the standard way in the system Vacuette ®.

Conclusion: Although the TLRs are important components of innate immunity in the framework of this study neither significant differences in the expression of mRNA nor protein in monocytes AD patients and healthy controls were observed. Decreased numbers of CD14++ and CD14 + monocytes in AD and T2D patients and vice versa significantly higher expression of CD14 protein in these groups compared to controls indicates that just monocytes could play an important role in the pathogenesis of diabetes. By comparing the mRNA expression of buffy coats of healthy blood donors and healthy controls we found significant differences in the expression of PRL, TLR2 and TLR4, and therefore we think that it is inappropriate to use buffy coats as healthy controls.

Key words: diabetes, monocytes, innate immunity, Toll-like receptors, prolactin