Abstract

Introduction: Type 1 diabetes mellitus is a chronic metabolic disease caused by autoimmune destruction of pancreatic beta cells. The theory of the disease onset is derived from study of a disease course in non-obese diabetic (NOD) mice, in which the diabetes occurs due to a dysregulation of the immune system. Experimental and clinical studies showed that the autoimmunity may be abrogated by immune intervention, which if initiated early enough may at least slow down the ongoing beta cells lost and preserve residual insulin secretion. But immune intervention alone is not sufficient to restore normoglycemia in the majority of cases. Several interventional studies showed that stimulation of proliferation and/or regeneration of beta cells are necessary to restore normoglycemia in animal models.

Aim of the study: To find out, if the combination of a potent immunosuppression (murine anti-thymocyte globulin (mATG), gusperimus) together with stimulation of islet regeneration (sitagliptin) will be able to slow down or reverse the course of the disease. Another aim is to identify the mechanism by which the substances act.

Material and methods: All experiments were performed in female NODShiLtJ ($H2^{g7}$) mice. The following parameters were examined at day 0, 7, 14 and 28: blood glucose, subpopulations of T-lymphocytes by flow cytometry, islet inflammation and presence of endocrine cells by immunohistochemical examination of pancreatic sections. Concentration of dipeptidyl peptidase IV (DPP-IV) and transforming growth factor β (TGFβ) in blood, gene expression profile from splenocytes and intraperitoneal glucose tolerance test.

Results: In the mATG group the remission occurred in 3 out of 28 mice. In the gusperimus, sitagliptin and in the diabetic control group the remission occurred in one case each. In the combined group the remission was not achieved in any case. The only effect of therapy on regulatory T-lymphocytes values was recorded in the mATG group, where a marked increase from 8.8 (6.2 – 10.0) % at day 0 to 14.9 (12.3 – 23.4) % at day 7 was present; $P < 0.01$. At the same time dropped in the mATG group values of CD8+ T-lymphocytes from 16.7 (10.9 – 21.9) % to 4.9 (1.8 – 8.5) %; $P < 0.001$. The concentration of DPP-IV was at the end of the study highest in the gusperimus group and made 885.3 (183.4 – 2175.4) ng/ml, the lowest concentration was in mice with remission and made 321.8 (277.0 – 737.5) ng/ml with significant difference between those two groups; $P < 0.01$. TGFβ concentration was highest in mice with remission in which represented 81172.0 (66779.9 – 132703.9) pg/ml and was significantly different from the sitagliptin group in which the concentration TGFβ was the lowest and made 36670.2 (4627.5 – 64787.5) pg/ml; $P < 0.001$.

Conclusion: In the case of overt diabetes none of the therapies seemed to be efficient to cure or at least slow down the course of the disease. Neither the combination of two diversely acting substances did lead to better results. Remission of diabetes was very rare and was accompanied by lowered concentration of DPP-IV and higher concentration of TGFβ in blood. Administration of sitagliptin let to a decrease of TGFβ values. Only the mATG administration had positive influence on T-lymphocytes subpopulations. Gusperimus, on the other hand, kept the inflammatory process in active phase.

Key words: Type 1 diabetes, NOD mouse, DPP-IV, TGFβ, mATG, gusperimus, sitagliptin, Tregs, autoimmunity