Abstract

This thesis explores the effects of low-carbohydrate (LC) diet on development of T1D, insulitis and alterations of immune parameters using spontaneous diabetes model, specific pathogen-free (SPF) non-obese diabetic (NOD) mice, when introduced from the age of 4 weeks or from *in utero*. The study includes glycaemia screening for evaluation of T1D incidence in NOD mice, insulitis scoring, cell staining and flow cytometry analysis focused mainly on FoxP3+Tregs, CD4+IL-10+, Tr1, potentially immunoregulatory γδ T cells, and CD8+ memory/effector T cells in mucosal and non-mucosal lymphoid organs.

We did not observe any significant effect of LC diet on diabetes incidence when it was introduced to NOD females from the age of 4 weeks. In line with these data, we found no substantial differences in cell subsets percentages in mucosal (mLNs, pLNs, PPs) and non-mucosal (spleen, iLNs) lymphoid organs in 12-week-old NOD mice fed with LC diet from the age of 4 weeks. This finding was reflected by no substantial differences in insulitis scoring in pancreata of NOD mice fed LC and standard diet from 4 weeks of age.

When we investigated the effects of LC diet introduced to NOD females from *in utero*, we obtained an unexpected and interesting finding of statistically significantly accelerated diabetes incidence in NOD mice fed with LC diet versus standard diet, which was confirmed by three independent experiments. This was reflected by changes in immunoregulatory T cell subsets in LC diet group, with a statistically significantly decreased CD4+IL-10+ T cells (spleen, mLNs), Foxp3+ Tregs (spleen, iLNs), Tr1 cells in the spleen; the percentage of γδ T cells was also decreased in the spleen. CD8+ central memory T cells were generally increased on the LC diet introduced from in utero (spleen, pLNs and iLNs). Reciprocal decrease was found in CD8+ effector T cells, possibly reflecting an accelerated migration of effector T cells to the pancreas in mice fed with LC diet from in utero compared with mice kept on a standard diet. This was consistent with the insulitis scoring in panreata of NOD mice fed with LC and standard diet from *in utero*.

Thus, this study brings new findings about the influence of LC diet on immunoregulatory and effector T cell subsets and acceleration of T1D when it is introduced to NOD mice from in utero. A very rare acceleration of the development

of T1D in NOD mice by an environmental factor – the LC diet, may shed more light on the pathogenesis of the disease. Further multidisciplinary experiments are needed to elucidate the complex effects of LC diet on developing immune system in NOD mice.

Key words: T1D, low-carbohydrate diet, NOD mice, Tregs, Tr1 cells, γδ T cells, effector T cells; memory T cells.