Tregitopes as a novel immunoregulators of pregnancy tolerance in mouse abortion prone model

An essential condition for the development of a healthy pregnancy is the establishment of correct interactions between the mother and the foetus. The presence of the allogeneic foetus induces state of the immune tolerance that allows embryo to implant and later the foetus to develop. The loss of immune tolerance to foetal antigens may result in reproductive failure. The disturbed mechanisms of immune tolerance may cause pregnancy disorders such as miscarriage and pre-eclampsia. The regulatory T lymphocytes (Tregs) are critical for establishing immune tolerance to the foetal antigens. It has been proved that, both in humans and mice, there is an increase of Tregs during normal pregnancy and decrease in their number and activity in spontaneous abortion cases. Moreover, the adoptive transfer of Tregs mitigates pregnancy loss in abortion-prone mice. It has been shown that short peptides found in human and mouse immunoglobulins (IgGs) may induce Tregs expansion. These natural Treg epitopes, named tregitopes are highly conserved and have high binding affinity to human class II major histocompatibility complexes.

The doctoral thesis aimed to 1) identify novel non-IgG source tregitopes that can bind to MHC II with high affinity and promote expansion of Tregs *in vitro*; 2) investigate the role of designed SGS and LKD peptides and mouse tregitope 167 and 289 in alleviating immune imbalance and preventing pregnancy failure in an abortion-prone mouse model.

In the first paper, C57BL6Foxp3^{GFP} mouse naïve T cells were co-cultured with antigen presenting cells (APCs) under stimulation of selected peptides and known tregitopes. Then, the frequency of $CD4^+CD25^+Foxp3^+$ and $CD4^+CD25^+Foxp3^+IL\text{-}10^+$ cells were analyzed using flow cytometry. In the second and third paper, CBA/J female mice mated with DBA/2J male mice were intraperitoneal injected with 100 μg tregitope 289 or 100 μg tregitope 167 or 100 μg SGS tregitope or 100 μg LKD peptide or 150 μL phosphate-buffered saline (PBS; vehicle) on day 0 post-coitum. On the fourteenth day of pregnancy the number or resorbed and viable embryos were evaluated. On the third day and fourteenth day of pregnancy, the T-helper cell 1/T-helper cell 2-related cytokine levels were measured using enzyme-linked immunosorbent assay. Additionally, the proportions of T and regulatory B lymphocytes, and the expression of costimulatory molecules (CD80, CD86, CD40) and major histocompatibility class II molecules on APCs were examined using flow cytometry.

In the first paper, it was shown that some of the *de novo* designed peptides exhibited tregitope properties and stimulated the development of Treg lymphocytes in the splenic lymphocyte population. In the second and third paper, it was demonstrated that the administration of mouse tregitope 167, 289 and newly designed SGS tregitope resulted in a statistically significant reduction in the foetal death rate compared with the control group. Flow cytometry analysis demonstrated that administration of either tregitope significantly increased the splenic pool of Tregs at the preimplantation stage of pregnancy. We also demonstrated that newly designed SGS tregitope and mouse 167 and 289 tregitopes downregulated the expression of the CD80 or CD86 costimulatory molecule of antigen-presenting cells, respectively. Additionally, SGS tregitope upregulated the serum levels of interleukin 2 and interleukin 10 at the preimplantation stage of pregnancy.

The performed *in vitro* studies identified two novel tregitopes. Moreover, the *ex vivo* studies demonstrated that treatment with known 167 and 289 tregitopes and novel SGS tregitope significantly increased the frequency of Tregs, enhanced the production of IL-10 by Tregs and changed the costimulatory phenotype of APCs, contributing to improved pregnancy outcome. Moreover, we confirmed that known tregitopes 167 and 289 and novel SGS tregitope have a beneficial effect on pregnancy outcome by limiting the foetal death rate. Tregitope-mediated immunomodulation can be a potential therapeutic strategy for immune dysregulation-induced pregnancy failure.