ImmunoTools *special* Award 2025

Laurenz Wolner, PhD-student

Supervisor: Assoc. Prof. Nina Pilat-Michalek, PhD

Center for Biomedical Research and Translational Surgery, Währinger Gürtel 18-20, 1090 Vienna, AUSTRIA

The potency of Interleukin-2 complex treatment for prevention of humoral rejection

The primary aim in preventing graft rejection is the induction of graft specific immunological tolerance. While current immunosuppressive regimens significantly increase short term graft survival, they come with an impaired general immune response, risk of infections etc. Additionally, while acute organ rejection can be prevented relatively well, immunosuppressive therapy does not impede chronic rejection and late graft loss, which remains to be a serious clinical problem. Chronic antibody mediated rejection is one of the leading causes for late graft failure and until now, there is no specific treatment. It was shown, that T-regulatory cells (Tregs) are crucial for suppression of auto- and alloimmune responses and that expansion of Tregs can induce immunological tolerance. While adoptive Treg transfer still presents with limitations, like the high costs for *ex-vivo* preparation, *in-situ* expansion of Tregs seems to be a feasible approach for reducing costs and increasing practicability. Additionally, the contamination of suppressive Tregs with non-Tregs and the lack of cell-specific surface markers for Tregs is a common problem that is related to *ex-vivo* expansion and can be circumvented with an *in-vivo* approach. Selective expansion of Tregs can be achieved with a specific Interleukin-2-based treatment (IL-2 complexed to a specific antibody against IL-2 (JES6-1) = IL-2 cplx).

Recently, our group could show that combining IL-2 cplx with rapamycin and anti-IL-6 significantly increases fully mismatched skin graft survival without the need for immunosuppression. The aim of my research will be to investigate the therapeutic capacity of IL-2cplx to prevent acute and chronic humoral rejection of cardiac allografts in a mouse

model of heterotopic cervical heart transplantation and the role and relevance of impaired humoral response for long-term graft survival. Additionally, we want to investigate the effect of IL-2 cplx treatment on B and T cell subpopulations relevant for transplant rejection (Immunophenotyping and memory response).

We will employ multi-color flow cytometry analysis to assess various immune cell types extracted from the spleen, lymph nodes and bone marrow, which are recognized for their significant involvement in tolerance induction. We will especially focus on GC B-cells, naïve B lymphocytes, marginal zone B-cells, T follicular regulatory cells, T follicular helper cells, graft infiltrating lymphocytes, plasma cells, NK cells, CD8 T cells and Tregs. T and B cell subsets will be assessed with the use of antibodies to detect cells with markers indicating antigen exposure or cells displaying markers for homing to lymphoid tissue. Furthermore, we will use markers indicating the phenotype of activated Tregs. To name a few: CD45R/B220, CD23, CD1d, CD138, CD95, CD3, CD4, CD185, CD44, CD25, FoxP3, MHC-I, CD279, CD274. Furthermore, we will investigate the role of Tregs in inhibiting immune response by using **anti-CD25 mab** for Treg depletion. In those mice, rapid graft rejection would indicate a pivotal role of Treg for prolonged graft survival.

Additionally, we use flow cytometry crossmatch for detection of donor-specific antibodies. Binding of serum Ig Abs to thymocytes is analyzed using fluorochrome-labeled anti-mouse IgG and IgM antibodies.

Hence, the requirement for a diverse array of antibodies is essential to delineate interacting cells through the expression profiling of specific markers, and it is evident that the utilization of ImmunoTools would significantly contribute to the formulation of distinct staining panels.

The assistance of ImmunoTools would be immensely valuable to analyze the effects of IL2-cplx treatment on Tregs and other cell types and thereby unveil molecular mechanisms of tolerance induction.

ImmunoTools *special* AWARD for **Laurenz Wolner** includes 7 reagents

FITC - conjugated anti-mouse CD62L, CD45R

PE - conjugated anti-mouse CD161

APC - conjugated anti-mouse CD11b, CD25, CD44,

functional antibody against mouse CD25 (IL-2R) DETAILS more AWARDS