ImmunoTools *special* Award 2024

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Mechanisms of regulatory T cell induced transplantation tolerance

Despite significant progress in the field of solid organ transplantation, chronic rejection remains one of the major challenges limiting long-term graft acceptance. While 1-year survival has improved significantly in recent decades, there has been only little improvement in long-term survival. Reasons for late graft loss or increased mortality are mainly progressive coronary artery disease (transplant vasculopathy, CAV) as well as renal failure and malignant tumors as side effects of chronic immunosuppression.

Therefore, induction of immunological tolerance defined as long-term acceptance of allografts without the requirement of lifelong immunosuppressive medication remains the holy grail in transplantation. Recently, regulatory T cells (Tregs) have become the focus of transplantation research due to their efficacy in suppressing autoimmune and alloimmune responses and their important role in maintaining self-tolerance. The ability of Tregs to prevent acute and chronic allograft rejection has already been demonstrated in numerous preclinical small animal studies.

The recent development of a simple but elegant approach facilitating IL-2 complexed to an anti-IL-2 mAb (IL-2cplx) enabled selective and effective stimulation of Tregs *in vivo*. Here, the anti-IL-2 clone JES6-1 is complexed with the cytokine IL-2 thereby selectively presenting IL-2 to cells with high-affinity IL-2 receptors, such as Tregs. Using this approach, it is possible to overcome challenges of to date Treg therapy including the lack of clear Treg surface markers to differentiate specific Treg subpopulations, the relatively low baseline numbers *in vivo*, and the limited availability of GMP-compliant procedures for the required sterility, identity, purity, and potency of a cell therapy product. Although pre-clinical studies already demonstrated that IL-2cplx are sufficient for long-term lung allograft acceptance and can induce tolerance towards fully mismatched islets, this could not be achieved in a stringent skin allograft model. However, we recently succeeded in developing a protocol by

synergizing the *in situ* expansion of Tregs by IL-2cplxs with rapamycin and anti-IL-6, which resulted in significantly prolonged survival of skin allografts. Notably, impairment of donor-specific antibody production and lack of recipient sensitization after skin graft rejection were also observed.

Although prolonged survival of fully mismatched skin grafts was achieved, all grafts were eventually rejected, raising the question of which cell subsets are responsible for long-term graft acceptance and loss of function. To answer this specific question, we aim to characterize graft infiltrating leucocytes as well as phenotype immune cells responsible for humoral and cellular rejection within secondary lymphoid organs at defined timepoints following transplantation/rejection utilizing flow cytometry. For this analysis antibodies defining T cell activation and memory status (CD4, CD44, CD62L, CD25, CD154) as well as other markers determining immune cells with importance in graft acceptance/rejection such as antigen presenting cells, myeloid and B cells (CD80, CD86, CD19, CD11b) would be of great significance. Furthermore, we want to verify the status of recipient sensitization in a mixed lymphocyte reaction where recipient splenocytes or lymphocytes are co-cultured with irradiated donor cells to detect potential donor-specific proliferation of recipient T cells. To verify proliferative capacity of responder cells, incubation of recipient cells with purified CD3ε is an essential positive control.

Thus, receiving the ImmunoTools Award would be of great importance for the development of different staining panels that allow the characterization of a variety of immune cells and would enable the determination of donor-specific recipient T cell sensitization which is of great importance in transplantation.

In summary, the main goal of this project is to unravel the molecular mechanisms of IL-2 complex-based Treg-mediated tolerance induction to significantly improve the long-term survival of fully mismatched grafts. I am confident that with the great support of ImmunoTools, I will be able to expand the knowledge of cellular and humoral rejection in our transplant model for subsequent translation to the clinic and benefit for transplanted patients.

ImmunoTools *special* AWARD for **Romy Steiner** includes 10 reagents

FITC - conjugated anti-mouse CD154

PE - conjugated anti-mouse CD4, CD25, CD44, CD80

PerCP - conjugated anti-mouse CD

APC - conjugated anti-mouse CD11b, CD19, CD62L, CD86

anti- CD3ε purified

DETAILS more AWARDS