ImmunoTools *special* Award 2018

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Manipulation of the T cell compartment with IL2mAb complexes for tolerance induction

Induction of immunological tolerance remains the `Holy Grail´ in transplantation medicine. Although during the past two decades, immunosuppressive therapy has greatly improved the short-term graft survival, long-term outcomes are far from satisfactory. The reduction of the activation and efficacy of the immune system - the main challenges faced by life-long immunosuppressive therapy - leads to an increased risk of opportunistic infections and secondary malignancies. Therefore, to remove immunosuppressive therapy, there is a pressing need to develop new treatment strategies to eliminate pathogenic responses to donor-antigens while preserving normal immune function.

A promising approach for the realization of a potential alternative therapy for tolerance induction is the use of $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells (Tregs). Although adoptive Treg transfer represents an exciting immunotherapeutic strategy, barriers to clinically feasible Treg immunotherapy involve stability, off-cell effects, and demonstration of cell preparation purity and potency. A very promising approach to overcome the risks associated with *ex vivo* manipulation and transfusion, describes the selective expansion of Treg cell numbers in situ, using IL-2/mAb complexes. Interestingly, manipulation of the T cell compartment with IL-2/mAb complexes has been demonstrated to induce long-term survival in full major histocompatibility complex (MHC) disparate islet allografts, but no remarkable effects have been followed to induce tolerance in the skin graft model.

The development of a new protocol in our group, combining IL-2/mAb with rapamycin and anti-IL-6, has been shown to result in a marked prolongation of skin graft survival. Based on these encouraging findings, the overall goal of my project is to investigate the mechanisms of IL-2 cytokine/antibody complex mediated transplantation tolerance in a clinical and immunological relevant model, for novel approaches to achieving transplantation tolerance that maximise graft survival and minimise adverse effects of immunosuppressive drugs.

We use FACS analysis for the characterization of different immune cell types from spleen, lymphnodes and skin that are known to play an important role in tolerance induction. For that we use the hallmark antibodies for T-cells (CD3, CD4, CD8,

CD45). More in detail, we will use antibodies to distinguish cells that reveal antigen exposure (CD45RA, CD45RO), from cells that express markers for homing to lymphoid tissue (CD62L, CD103, CCR7), or markers known to be expressed upon activation (CD25, CD69, CD154). Moreover, we will use as well markers for the phenotype of activated Tregs, namely CTLA-4, GITR, CD25 and FoxP3^{+}. Additionally, we will focus on antigen presenting cells and the expression of relevant markers for co-stimulation such as CD80 and CD86, just to name a few.

Therefore, a broad range of different antibodies is needed to characterize interacting cells by the expression of certain proteins and ImmunoTools for sure would be of great help to develop different staining panels. Furthermore, suppressive or inflammatory properties within the complex immune environment of different leukocytes will be analyzed using ELISA (eg. IL-6, IL-17A and TNF-alpha)

To summarize, the aim of my project is on the one hand to find a treatment strategy that successfully delays skin graft rejection based on the selective expansion of Tregs with IL-2 cplx treatment, and on the other hand to unveil a better understanding of the molecular mechanism in tolerance induction. I am sure with the great support of ImmunoTools I will expand my knowledge about the cellular mechanism in this model.

ImmunoTools *special* AWARD for **Mario Wiletel** includes 25 reagents

APC – conjugated anti-mouse CD11a, CD11b, CD19, NK-cells

FITC - conjugated anti-mouse a/b TCR, g/d TCR

PE - conjugated anti-mouse CD34, CD54, CD80

recombinant human cytokines: rm IL-22, rm IL-33, rm GRO-a/ CXCL1, rm MIP3b / CCL19

mouse ELISA-set (for one 96 plate): mouse IL-6, mouse IL-17A, mouse TNF-alpha

DETAILS more AWARDS