ImmunoTools *special* Award 2016

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Characterizing neo-antigen specific immune responses in a skin gene therapy setting

I am a 1st year PhD student working on a project that aims to elucidate the regulatory mechanisms governing immune homeostasis within human skin with the goal to understand and establish long-lasting immune-tolerance towards new skin antigens *in vivo* (in a skin gene therapy setting.)

I am working in close collaboration with the only specialized clinic worldwide for treatment and research of the hereditary skin blistering disease Epidermolysis bullosa (EB; “butterfly children”). EB is caused by mutations of one of several structural skin proteins, and all causative therapies aim at introducing the correct skin protein into the patient's skin. As the patients often completely lack the wild type form of the structural protein, the therapy will lead to the presence of an unknown (i.e. “non-self”) protein antigen in the patient’s skin.

It has previously been shown that neo-antigens in the skin induce an inflammatory reaction. Based on these data it is thought to be likely that the patient’s immune system will equally recognize this new gene-therapeutical antigen as “non-self” and as a consequence will mount an immune response against the new skin protein, leading to the rejection of the corrected skin. These facts made me deeply appreciate the need to understand and manipulate immune-regulatory mechanisms acting in human skin.

Neo-antigens introduced into peripheral tissues, such as the skin, are not present in the thymus and we therefore aim at employing peripheral (and not thymic) regulatory mechanisms to prevent rejection. Suppressive, peripheral Treg develop from naïve $CD4^+$ T cells when they encounter their cognate antigen in the correct immunological context. We hypothesize that enhancing regulatory T cell (Treg) numbers and function in the skin could serve as therapeutic approach to establish stable tolerance to the introduced neo-antigens.

In order to test this hypothesis we will develop a humanized mouse model for EB skin therapy, in which gene corrected human skin and the patient's immune cells are transplanted to immuno-deficient NSG mice. In the future this model will serve as a potent tool to study and manipulate the tissue-specific immune response towards neo-antigens.

In detail, the patient's PBMCs will be adoptively transferred to NSG mice that have previously been grafted with human skin containing the gene therapeutically altered skin proteins (i.e. the neo-antigen). We will subsequently follow the neo-antigen specific immune response and try to promote the generation of pTreg and thereby induce tolerance to the neo skin proteins (i.e. by administration of rh IL2 and rh IL7). Additionally we will use *in vitro* generated tolerogenic DCs, to further manipulate the immune response towards the neo skin antigen *in vivo.*

The development of this model requires the characterization of the EB patient's immune status prior to the *in vivo* studies in the humanized mouse model. For that we will isolate PBMCs from patient's blood and phenotype the leukocyte population using flow cytometry. Additionally, the inflammatory properties of the leukocytes will be characterized using ELISA. ImmunoTools reagents, such as TGF-β, Defensin-β and IL-22, IL-17 and IFN-γ, will be used to culture human immune cells *in vitro*, differentiate dendritic cells *in vitro*, test the effects of various pro- and anti-inflammatory cytokines in *in vitro* and *in vivo* wound healing assays (using co-cultures of PBMCs and primary keratinocytes and/or fibroblasts of patients) and treat humanized mice with cytokines *in vivo*.

With this model we will create a unique tool to study the generation of long-lasting antigen-specific immune tolerance and immune homeostasis in the skin. This award would therefore support me in evaluating some of the immunological questions that still remain unanswered.

ImmunoTools *special* AWARD for **Maria Klicznik**

includes 21 reagents

FITC - conjugated anti-human IL-6

PE - conjugated anti-human TNFa

APC - conjugated anti-human CD11b, CD69

recombinant human cytokines: rh Defensin-beta, rh GM-CSF, rh IFN-gamma, rh IL-1alpha, rh IL-2, rh IL-4, rh IL-7, rh IL-10, rh IL-13, rh IL-17A, rh IL-22, rh MCP-1 / CCL2, rh MIP-1b / CCL4, rh sCD40L / CD154, rh TGF-beta3, rh TNFα, rh VEGF-A/VEGF-165

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