ImmunoTools *special* Award 2014

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The role of the transcription factor STAT5 in murine Natural Killer cells

Natural Killer (NK) cells are innate lymphocytes developing from a common lymphoid progenitor in the bone marrow. They represent the first line of defense against microbe-infected, stressed and malignant cell. To fulfill their missions NK cells are potent cytokine producers, express ligands for death receptors, release lytic granules and are equipped with numerous activating and inhibitory receptors. NK cell functions are controlled by several cytokines and subsequently JAK-STAT signaling. STAT1 is known to regulate NK cell cytotoxicity and cytokine production. STAT4 is highly expressed in resting NK cells and regulates IFN-γ production. STAT6 has been reported to be involved in the differentiation of NK cells. By generating the first Cre recombinase system that specifically deletes in the NKp46^{+} NK cell compartment (Ncr1-iCreTg mice) our group investigated the role of the transcription factors STAT3 and STAT5 in detail. We have recently shown that NK cells display an enhanced cytotoxicity and improved tumor surveillance in the absence of STAT3. Using Stat5Δ/ΔNcr1-iCreTg mice, we could proof that STAT5 is absolutely necessary for NK cell survival.

IL-15 is the most prominent cytokine in NK cell biology and signals predominantly via the transcription factor STAT5. The fact that NK cells fail to develop upon lack of STAT5 forced us to find another tool to study its function in NK cells. By introducing another mouse strain, we discovered that the overexpression of Bcl-2 suffices to restore NK cell numbers in Stat5Δ/ΔNcr1-iCreTg mice. This allowed us to investigate the impact of STAT5 transcription for NK cell function. Our data unravel STAT5 as the master regulator of NK cell biology. We discovered that besides NK cell

development and survival, STAT5 controls NK cell maturation, induces proliferation and is involved in the regulation of the lytic machinery and NK cell effector functions. Studying NK cell cytotoxicity using different *in vivo* models revealed unexpected consequences for NK cell dependent tumor surveillance. We are currently investigating further mechanistically details.

Future experiments should as well dissect STAT5A and STAT5B dependent effects and concentrate on the upstream and downstream signalling involved in our findings.

Thus the selected cytokines and antibodies from Immunotools would be extremely helpful to perform further flow cytometric analysis and to investigate the signaling cascade in more detail. Additionally, we could use the selected antibodies (anti human CD3, CD16, CD56 and CD57) to detect and sort through flow cytometry human peripheral blood NK cells. Thereby we could establish a new tool in our laboratory and could test the human relevance of our findings. This would increase the strength of our findings linking the animal data to the human system.

With the experiments mentioned above, we could set the focus on *in vitro* assays and thus reduce the number of animal experiments that is in accordance with the 3R principle. We hope you consider our work as adequate and are looking forward to use your tools.

ImmunoTools *special* AWARD for **Dagmar Gotthardt** includes 22 reagents

FITC - conjugated anti-human CD3, CD16, CD56,

PE - conjugated anti-human CD57,

PerCP - conjugated anti-human CD3,

APC - conjugated anti-human CD3, CD16, CD56,

recombinant human cytokines: rh IL-15,

FITC - conjugated anti-mouse CD3e, CD11b, Gr-1, NK-cells,

APC - conjugated anti-mouse CD3e, CD4, NK-cells,

recombinant mouse cytokines: rm GM-CSF, rm IFNgamma, rm IL-2, rm IL-6, rm IL-15, rm VEGF

DETAILS more AWARDS