ImmunoTools *special* Award 2014

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Nlrc5 is a major transcriptional regulator of MHCI

In our lab, we are interested in studying the NOD-like receptor (NLR) family member CARD containing 5 (NLRC5). For this purpose, we generated and studied the phenotype of *Nlrc5-deficient* mice (Staehli et al., 2012, The Journal of Immunology). We found that lymphocytes derived from *Nlrc5* knockout mice displayed dramatically reduced expression of major-histocompatibility complex (MHC) class I in lymphocytes and intermediately decreased levels in myeloid cells. MHC class I is a crucial molecule of the immune system as it enables the body to recognize infected or tumor cells and to destroy them. Interestingly, the reduced protein expression in *Nlrc5-deficient* cells was paralleled by markedly decreased MHC class I gene transcript levels. We could additionally show that endogenous NLRC5 efficiently occupies the promoter of these genes and thereby regulates MHC class I transcription, thus fulfilling a key transcriptional regulatory function within the adaptive immune system.

The crucial role of NLRC5 in controlling MHC class I expression amongst immune cells renders this protein a particularly exciting subject of research with regard to immunity and immune disorders, including autoimmunity and transplant rejection problems. Presently, however, not much is known neither on the molecular mechanisms by which NLRC5 operates, nor about its regulation.

One major part of our future work will address the transcriptional and post-translational regulation of NLRC5 in lymphocytes, as well as myeloid-derived antigen-presenting cells. In fact, we have evidence that NLRC5 is subject to multilevel regulatory networks, encompassing transcriptional and complex posttranslational mechanisms (Staehli et al., 2012, The Journal of Immunology).

For this purpose, we plan to isolate the different lymphocyte subsets by fluorescence-activated cell sorting (FACS)-sorting. For these experiments, the following anti-mouse antibodies for flow cytometry will be very useful: CD3e, CD4, CD8a, CD11b, CD19, CD44, CD62L, NK-cells. Concerning the more rare myeloid subsets, we foresee to differentiate these cells in vitro from bone-marrow progenitors. To this end, recombinant cytokines and growth factors from ImmunoTools will be used to differentiate bone marrow cells into macrophages (M-CSF), conventional dendritic cells (GM-CSF + IL-4) and plasmacytoid dentritic cells (Flt3L).

FACS-sorted lymphoid cells and in vitro-differentiated myeloid subsets will be cultured – and in certain cases expanded – in vitro within the appropriate cytokine milieu. The cells will then be and stimulated with a panel of microbial products, pro-inflammatory and regulatory cytokines (IFNgamma, rm IL-2, rm IL-7, rm IL-10, rm IL-15, rm IL-22, rm IL-33). At different time points following the stimulation, the cells will be harvested and analyzed by quantitative PCR in order to measure *Nlrc5* transcription and by western blot to assess posttranslational modifications of NLRC5.

These experiments will allow us to gain a comprehensive view on different mechanisms controlling NLRC5 expression and activity. These experiments will therefore pave the road to further analysis aimed at dissecting the functional contribution of such regulatory aspects in vitro and in vivo.