ImmunoTools *multiplex* Award 2016

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Activation and ex vivo expansion of human T lymphocytes

T Lymphocytes, also called T cells, belong to the adaptive immunity of our body's defense system. αβ T cells recognize foreign antigens, such as those derived from viruses or bacteria, presented on MHC molecules. In contrast, γδ T cells recognize stress-induced antigens and small phosphorylated compounds collectively called phosphoantigens. This recognition is accomplished by the αβ and γδ T cell antigen receptors (αβ TCR and γδ TCR), respectively, that are expressed on the surface of these immune cells. Initially, T cells are naïve T cells that are not further differentiated. However, when being stimulated by antigens they become activated and differentiate to various effector cell types. This differentiation depends on additional signals, such as cytokines. As effector cells they migrate to the site of inflammation to combat the incoming antigen. Our group is interested in the molecular mechanisms that govern these activation and differentiation events.

In particular, our laboratory is interested in the signalling events and expression pattern of signalling proteins and related proteins as the T cells are activated and differentiate. We use human αβ and γδ T cell lines as well as cultures of primary αβ and γδ T cells isolated from the blood of healthy donors. In the past our group has focussed on the TCR itself. We could show that the αβTCR can form antigen-independent nanoclusters on the surface of T cells and that these nanoclusters are involved in regulating the sensitivity of the T cell activation event towards antigenic stimulation. Further, we could show that the αβ TCR undergoes a conformational change at its CD3 subunits upon antigen-binding. This CD3 conformational change is crucial for CD3 phosphorylation and thus is an important event in αβ T cell activation. Interestingly, this conformational change is neither occurring in γδ TCR nor is it required for γδ T cell activation. Thus, γδ T cells differ from αβ T cells in a so far unappreciated manner. Now we want to expand our interest from the TCRs

themselves to signalling proteins and proteins that regulate the activation and differentiation process of these cells.

In this project, we plan to further characterize different activation and differentiation states of αβ and γδ T cells. One focus will not only be the side-by-side comparison of unstimulated versus stimulated cells, but also the comparison of αβ T cells with γδ T cells. The Multiplex Array will be an instrumental tool to reach this goal in an efficient manner. We will use expanded T cells from human blood using different protocols for their generation as well as unstimulated and TCR-stimulated T cells. These different populations will be compared by the Multiplex Array. Furthermore, this ImmunoTools award will help us to establish this modern technique in our lab in Freiburg.

ImmunoTools *multiplex* AWARD for Omid **Sascha Yousefi**
includes free analysis of samples on several antibody arrays with large range of antibodies against human CDs, human cytokines, and others.