ImmunoTools *special* Award 2014

Janine D. Dreesen, PhD student

Supervisor: Priv.Doz. Dr. Bernhard B. Singer

Universitätsklinikum Essen (AöR), Institut für Anatomie,
Hufelandstraße 55 D- 45147 Essen

T cell activation: Mechanisms of CD3 driven T cell functions in health and cancer: long forgotten or astonishing new?

In the beginning of my PhD thesis I started to study different aspects of T cell proliferation triggered by different monoclonal antibodies (mAb) binding to CD3, alone or in combination with the well-known T cell co-stimulator CD28. In contrast to various other labs we decided to work with freshly isolated human PBMCs (peripheral blood mononuclear cells) instead of cell lines (e.g. Jurkat). Furthermore, we performed our assays on PBMCs instead of pure T cells to mimic conditions as physiological as possible. Usually, PBMC of healthy donors consist of about 5-15% B cells, 5-10% NK cells and 5-18% monocytes next to the lion's share of about 60-70% T cells. The different types of leukocytes are know to communicate and interact with each other and thus to influence cellular behaviour in response to stimulation. Our PBMC were stimulated with very low concentration of two different anti-CD3 mAbs applied soluble without additional co-stimulator.

While one of the anti CD3 antibodies induced T cell proliferation in cells of all donors, the other one revealed its mutagenic effect in PBMCs of just 50% of all donors. Comparing this effect in PBMCs of healthy donors and cancerous patients we found a clear difference with respect to the T cell response profile. Instead of 50% "Responders" (persons with T cells proliferating in response to anti CD3-stimulation) in healthy donors more than 70% "Responders" were found in patients suffering various types of cancer. Having tested hundreds of patients and healthy donors we recognized that a tiny amount of the so-called "Non-Responders" showed another remarkably feature. The cells of approximately 20% of the "Non-Responders" (up to now this is only true for cancer patients) showed a new distinct cell population as analyzed in flow cytometry. That means after taking the blood and isolating the PBMC, flow cytometric images showed a distinct population containing B and T lymphocytes together with NK cells as well as another smaller population of monocytes. After three days of cultivation untreated control cells looked rather similar to the ones at day zero except that 50% of the monocytes were lost in the measurement because of their adherence to the cell culture plates. If T cells proliferated through the CD3 stimulus, the monocytes were gone and the lymphocytes showed a characteristic banana-shaped form if analyzed in the FSC/SSC modus of the flow cytometer. For most of the Non-Responders incubation with that distinct CD3 antibody did not render cytometric images compared to the

control, but in 20% of the cases a new type of cell population appeared next to the monocytes. This population contained cells with the width of monocytes. First we thought that the monocytes proliferated, but BrdU Proliferation Assays showed that there is no proliferation within the PBMC. Therefore we hypothesised that these cells represent a matured or differentiated leukocyte subpopulation. With the selected ImmunoTools antibodies we plan to characterize these leukocyte subpopulation. The results will not only help us to identify the putatively novel cell population but also to find out more about how the CD3 antibodies trigger cell differentiation/maturation and the reason for what distinguishes people with and without this yet undefined cell population.

ImmunoTools *special* AWARD for **Janine D. Dreesen** includes 14 reagents

FITC - conjugated anti-human CD11b, CD14, CD40, CD43, CD45, CD45RA, CD61, CD80,

APC - conjugated anti-human CD3, CD4, CD8, CD11c, CD40, Annexin V,

DETAILS more AWARDS