ImmunoTools *special* Award 2021

Dr. Yazid J. Resheq, Clinician Scientist

Dept. of Internal Medicine I, University Medical Centre Ulm,
Albert-Einstein-Allee 23, 89081 Ulm, Germany

Role of cholesterol metabolism in T-cell mediated tumor rejection

Harnessing the immune system to fight cancer has proofed to be effective in modern medicine. In this context, immune checkpoint inhibitors (ICIs) have revolutionized immunotherapy. Hallmark of ICIs is that they prevent the silencing of T-cells, which are critical for tumor rejection, by binding to so called “checkpoint” molecules or their corresponding receptors (i.e. Atezolizumab, Pembrolizumab). However, response to ICIs vary amongst individuals and recent studies indicate that concomitant medication may affect treatment efficiency in not anticipated ways (i.e. intake of proton pump inhibitors) (1). Herein, immunometabolism deserves special attention, as interfering in the metabolism may not only affect the performance but equally the polarization of immune-cells. Whereas glucose metabolism has been studied at length, only few is known about the role of lipid metabolism in this context. However, cholesterol has not only a pivotal role in preserving cell integrity under physiological condition. In fact, early data show that cholesterol is required for T-cells, i.e. by supporting T-cell antigen receptor nanoclustering which is mandatory for their activation (2). Noteworthy, I was able to show that production of H_2O_2 and uptake of LDL is interconnected in monocyte-derived dendritic cells controlling distinct properties of this cell-type (3). Interestingly, recent findings indicate that cholesterol enhances T-cell-driven rejection of tumors in a murine model. Unfortunately, clinical data of the concomitant intake of medication interfering with the cholesterol-metabolism such HMG-CoA-reductase inhibitors (“statins”) in tumor-immunology (incl. individuals treated with ICIs) are contradictive (4) (5). Given the fact, that many cancer patients suffer from concomitant diseases requiring the intake of lipid-lowering drugs such as “statins” on the one hand and the variable response to ICIs on the other hand, the role of cholesterol-metabolism in such a scenario needs to be addressed.

The aim of the here proposed study is therefore to analyze the impact of agents interfering with lipid-metabolism such as “statins” on T-cell mediated tumor eradication in combination with ICIs.

For this purpose we will use T-cells from HLA-A2$^+$ healthy donors who have detectable antibodies against CMV. T-cells will then be cocultured with PD-L1$^+$ cell-lines of various tumors, which are HLA-A2$^+$ allowing the presentation of pp65-peptides. We will screen different cell-lines for HLA-A2 and PD-L1-expression. In cases where no PD-L1-expression can be detected we will use a CRISPR/CAS-based approach to express PD-L1. In cocultures, we will use commercially available anti-PD-L1 antibodies, which will be combined with different agents interfering with lipid-metabolism, most notably statins. Additionally, we will use an experimental ACAT-inhibitor, which has been shown to enhance cholesterol-concentration in the membrane of CAR-T-cells. At given time points, cocultures will be analyzed using flow-cytometry. This includes antigen-specific tumor killing by T-cells, polarization of T-cells, analysis of T-cell-activity such as proliferation or secretion of Granzyme B. In some experiments, T-cells will be re-challenged for the capacity to be activated following cocultures with tumor-cell-lines (i.e. antigen-independent proliferation using CD3/28-beads).

From the data deriving from this project I expect to identify potential targets to be analyzed in future studies. Hence, this award will give me the opportunity to generate important preliminary data to apply for further funding from research councils such as DFG.

Literature

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