ImmunoTools *special* Award 2021

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Functionalization of primary human T cells with superparamagnetic iron oxide nanoparticle (SPION) for magnetic cell targeting in anti-cancer immune therapy

The Section of Experimental Oncology and Nanomedicine (SEON) in the Department of Otorhinolaryngology, University Hospital Erlangen, develops superparamagnetic iron oxide nanoparticles (SPIONs) for biomedical applications and treatment of diseases such as cancer.

For tumor therapy, we are establishing the magnetic targeting of functionalized cells to increase the infiltration of immunologically “cold” tumors with cytotoxic T cells. For magnetic delivery of immune cells, primary human $CD3^+$ T cells are loaded with superparamagnetic iron oxide nanoparticles (SPION) to be applied into the vascular system of a tumor and enriched in the tumor region by an external magnetic field. Aim is to improve the immune cell infiltration of initially immunologically “cold” tumors, which is associated with a better clinical prognosis.

To make T cells controllable by an external magnetic field, the amount of SPION loading must be sufficient. We investigated the optimal SPION loading strategies for T cells, regarding experimental conditions such as cell culture medium, supplements, incubation time, and the stability of engulfed SPIONs. Incubating freshly isolated T cells with an iron concentration of 80 µg/ml overnight resulted in both SPIONs intracellularly in vesicles and SPIONs attached to the cells` plasma membrane, which was confirmed by transmission electronic microscopy. Furthermore, we showed that after SPION loading the T cells remained viable and effector functions were not impaired after polyclonal expansion. Importantly, the amount of engulfed nanoparticles was sufficient to enrich the cells magnetically on a specific position, even if the nanoparticle-loaded T cells were placed into a peristaltic pump, mimicking the tumor suppling vascular system (Boosz et al. 2021).

Our future goal is to transfer the successful *in vitro* cell culture model to an *in vivo* model. This challenging task needs studies on the antigen-specific antitumor response of the loaded T cells, on the behavior in vascular systems, on the capability of SPION-loaded T cells to perform tissue infiltration and on possible side effects regarding interaction with other cells and immune activation.

The reagents from the ImmunoTools special award will enable us to analyze the impact of SPION-loading of $CD3^+$ T cells regarding their effector functions after polyclonal and antigen-specific activation using flow cytometry.

To cultivate the freshly isolated human primary $CD3^+$ T cells, the recombinant ImmunoTools human cytokines rh-IL2 and rh-IL7 will be used. Their viability will be monitored via Annexin V-FITC.

For estimation and quantification of nanoparticle uptake, we will investigate the side scatter change in flow cytometry as well as analyze the cellular iron amount using atomic emission spectroscopy of the SPION-loaded cells.

To discriminate between the T cell populations the following ImmunoTools antibodies will be used: anti-CD3-FITC, anti-CD4-PerCP, anti-CD8-FITC.

Upregulation of activation markers after polyclonal and antigen-specific T cell stimulation will be determined via ImmunoTools anti-CD25-PE, anti-CD69-APC.

The SPION-loaded T cells will be analyzed regarding their effector functions. For intracellular cytokine staining, anti-IFNγ and anti-TNFα antibodies will be used.

Reference:

Boosz P, Pfister F, Stein R, Friedrich B, Fester L, Band J, Mühlberger M, Schreiber E, Lyer S, Dudziak D, Alexiou C, Janko C: Citrate-Coated Superparamagnetic Iron Oxide Nanoparticles Enable a Stable, Non-Spilling Loading of T Cells and Their Magnetic Accumulation. Cancers, 13, 4143, 2021

ImmunoTools *special* AWARD for **Philipp Boosz** includes 10 reagents

FITC - conjugated anti-human CD3, CD8, TNFα, Annexin-V-FITC

PE - conjugated anti-human CD4, CD25, IFNγ

APC - conjugated anti-human CD69

recombinant human rh IL-2, rh IL-7

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