P-088

Intracellular [^{64}Cu]PTSM and extracellular [^{64}Cu]DOTA-antibody labelling of ovalbumin-specific Th1 cells for *in vivo* PET investigations of Th1 cell trafficking in OVA-specific lung inflammation

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Introduction: T helper cells play an important role in the development of autoimmune diseases. For detailed in vivo analysis of the migration properties of Th1 cells, high sensitive imaging modalities, such as small animal PET are powerful tools. So far basic migration properties like kinetics, homing, and sites of T cell proliferation in animal models for autoimmune diseases are still poorly understood. The aim of our study was to establish new T cell labelling strategies to gain new insights in Th1 cell trafficking in vivo using small animal PET. In our studies Th1 cells were in vitro labelled intracellularly with the lipophilic tracer [^{64}Cu]PTSM or extracellularly with [^{64}Cu]DOTA-linked antibodies prior to injection into diseased mice and tracking by small animal PET.

Methods: To investigate whether intracellular [^{64}Cu]PTSM labelling or extracellular [^{64}Cu]DOTA-antibody labelling impair ovalbumin (OVA)-specific Th1 cells, we analysed cell viability and functionality. OVA-T cell receptor (TCR) transgenic CD4+ T cells were isolated from spleen and lymph nodes of DO.11.10 mice and cultured together with irradiated antigen presenting cells (APC), Oligo 1668 peptide, anti-IL-4, and IL-2 for 12-14 days. 10^6 OVA-Th1 cells were labelled with 0.7 MBq [^{64}Cu]PTSM for 3 hours or with 0.7 MBq radiolabelled OVA-TCR-specific antibody (KJ1-26), which was linked to [^{64}Cu] via the chelator DOTA, for 0.5 hours. Th1 cell viability was assessed by trypan blue staining after incubation with increasing amounts of activity. Specific Th1 cell functioning was analyzed through interferon-gamma (ELISA) levels in supernatants of specific activated OVA-Th1 cells (T cells + irradiated APC + OVA peptide). In vivo T cell migration was investigated in an animal model for OVA-induced lung inflammation. Mice were sensitized with OVA (i.p.) and challenged intranasally twice after four weeks to induce OVA-specific lung inflammation. A total of 10^7 [^{64}Cu]PTSM or [^{64}Cu]DOTA-KJ1-26 antibody labelled OVA-Th1 cells were injected i.p. into diseased and healthy mice. Static PET-scans in combination with CT, biodistribution, and autoradiography were performed 24 and 48 hours after OVA-Th1 cell transfer.

Results: In vitro investigations revealed an activity dependent impairment of OVA-Th1 viability and functionality after [^{64}Cu]PTSM or [^{64}Cu]DOTA-KJ1-26 antibody labelling. After a time period of 24 hours post labelling, the cell viability sunk to 80% and functionally was decreased by 20% compared to unlabelled control cells. Analyzing OVA-specific Th1 cell migration in the mouse model for lung inflammation, we detected an accumulation of [^{64}Cu]PTSM labelled OVA-Th1 cells in lung tissue and the thymus already 24h after the final challenge. Detection of [^{64}Cu]DOTA-KJ1-26 antibody labelled OVA-Th1 cells was even possible for up to 48 hours. In vivo PET data were further confirmed by ex vivo biodistribution and autoradiography. Compared to [^{64}Cu]PTSM labelled OVA-Th1 cells we gained a more defined distribution of extracellularly [^{64}Cu]DOTA-KJ1-26 labelled cells in the peritoneum (the site of injection), draining lymphatic tissue and the sites of OVA-specific lung inflammation. Furthermore we detected a significant accumulation of OVA-Th1 cells in the omentum majus.

Conclusions: Both [^{64}Cu]-based labelling methods cause an impairment of Th1 cells viability and functionality. However, both labelling strategies are applicable for in vivo PET-imaging, revealing a detection limit of 500 OVA-Th1 cells in draining lymph nodes and sites of OVA-specific lung inflammation over a time period of 48 hours.