IN VIVO IMAGING OF INTRACELLULAR GRANULE TRAFFICKING IN DENRITIC EPIDERMAL T CELLS

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Dendritic epidermal T cells (DETCs), a population of epithelial T lymphocytes bearing γδ T cell receptor (TCR), remain constitutively activated through synapse-like structures called phosphotyrosine-rich assemblies located on projections (PALPs) [1]. Similarly to the classical immunological synapse, which mediates directional trafficking and release of intracellular effector cargos and TCR recycling, PALPs contain lysomal and GM1 ganglioside-rich granules. It is not clear, however, whether the existence of granular structures in DETCs and PALPs is associated with a directional intracellular movement of the cargo.
To investigate the dynamics of DETC granules we developed a method of intracellular vesicle visualization and studied in vivo trafficking of intracellular cargo inside DETC bodies using intravital microscopy and fluorescence recovery after photobleaching (FRAP). The granules were labeled upon intradermal injection of Lysotracker Red DND-99 and Alexa Fluor (AF) dyes conjugated with cholera toxin subunit B (CTB) or α-TCR antibody. CTB-AF555 and α-TCR-AF647 antibody were internalized with different kinetics and several days after injection (up to 11 days) AF555- and AF647-positive granules could be still detected inside DETCs.

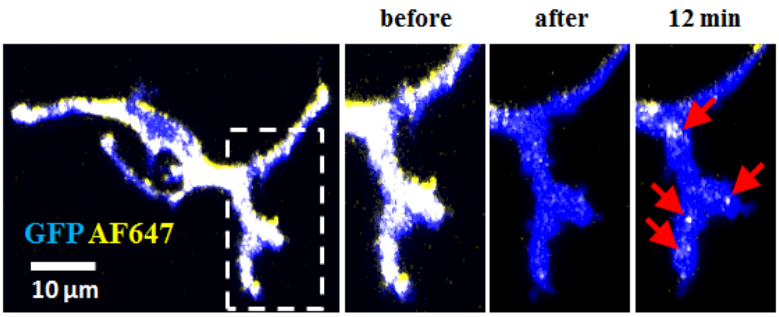

Fig. 1. Intravital imaging of granule dynamics. Image of a DETC (blue) loaded in vivo with α-TCR-AF647 antibody (yellow) 9 days earlier. Dashed line indicates photobleached area. The area was observed for additional 12 min. Red arrows point to arriving granules.

Imaging of granules inside DETCs was performed in vivo in ear skin of anesthetized IL2p8-GFP mice using a resonant scanning Leica SP8 microscope through a 40x oil objective. Granule-associated fluorescence was photobleached in sub-cellular areas of DETC bodies (dendrite tip, main cell body) and the kinetics of FRAP was analyzed. We found that whereas the apical dendrites remained stably anchored, the inside tubular lengths of these cellular projections were highly dynamic, trafficking at least two types of discrete cargo packets in opposite directions. Our results suggest that the localized self-reactivity of epidermal γδ TCR establishes a highly organized trans-epithelial conduit system for directional trafficking of granular cargo.

References: [1] G. Chodaczek et al., *Nature Immunol.* **13**, 272-282 (2012)

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