

IN VIVO INSPECTION OF EGFP LANGERHANS CELLS IN THE EPIDERMIS WITH 5D CONFOCAL MICROSCOPY.

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We wished here to visualize in living mice the mobility of epidermal Langerhans cells (LC) involved in the capture of pathogens, leading to their migration to draining lymph nodes and initiation of immune responses. Langerhans cells belong to the dendritic cells lineages and are located in the supra basal layer of the epidermis where they form a characteristic network with thin dendrites wrapped around keratinocytes. LC express major histocompatibility class II molecules and the C type lectin Langerin (CD207), which is present at the cell surface as well as in specific organelles referred to as Birbeck granules.

To monitor the migration properties of LCs, we engineered knockin mice that expressed under the control of the langerin gene an enhanced green fluorescent protein (EGFP). Comprehensive phenotyping of LCs found in epidermis and cutaneous-draining lymph nodes showed a faithful EGFP expression in all Langerin-positive cells. Using 5D intravital confocal microscopy of the superficial ear skin layers, we monitored the motility of epidermal resident Langerhans cells after application of various inflammatory stimuli. Using a recently developed tape stripping method to induce a mechanical disruption of the epidermal barrier and local skin inflammation, we found that LCs located next to the dermal-epidermal junction or next to hair follicles were motile over period of time of 30-60 min, whereas typical epidermal LCs with branched dendrites are immobile. We will present a new visualization method based on animated movies in RGB, which highlights the leading edge in red and trailing edges in blue and the main cell body in white in EGFP positive cells. These RGB movies reveal that mechanical stress of the skin layer induces high dendrite motility over long periods of time extending to up to one day.

Migrating LCs harbour a limited number of dendrites occasionally tethered to neighbouring structures. Precise three-dimensional examination of the EGFP labelled LCs in the epidermis revealed that their dendrites are remodelled after mechanical stress. We suggest that LCs can acquire a migratory phenotype *in vivo* following interaction with disrupted keratinocytes and are prone to sense danger signals from various pathogens entering the epidermis. This work represent the first intravital 5D confocal imaging of Langerhans cells of the skin and highlights their sustained reactivity to environmental stress in the skin.

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