**Keratinocyte-derived exosomes in shaping the tolerogenic phenotype of dendritic cell populations**

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Human epidermis, forming the outermost layer of the skin, is made up of epidermal keratinocytes. Being directly exposed to the external environment, those cells are the first point of contact for environmental allergens which, although harmless to healthy individuals, can spark an immune response in people with conditions such as skin allergies or atopic dermatitis. Keratinocytes have shown the ability to attract immune cells by chemokine secretion and activate them by released cytokines. In turn, those cells stimulate keratinocytes further, creating a positive feedback loop that sustains the inflammation. Apart from directly influencing the immune response by secreting signaling molecules, keratinocytes could potentially also contribute through the release of exosomes, extracellular vesicles about 30-150 nm in diameter. While studies in mice have shown that depending on the cytokine cocktail used to stimulate keratinocytes, exosomes derived from those cells varied in their content and showed the ability to alter the phenotype of bone marrow-derived dendritic cells, little is known about the immunomodulatory capacity of such exosomes in human system and with relation to atopic skin disease.

There are several types of immune cells, i.e. dendritic cells (DCs), that are encountered by antigens penetrating through the epidermis, including the subset of dendritic cells specific for this tissue, Langerhans cells (LCs). DCs are key for the immune response since they process and display antigens on their surface, which leads to activation of antigen-specific T cells which further enhances immune activation. However, upon exposure to tolerogenic signals induction of dendritic cells with suppressive phenotype (tolDC) occurs; these downregulate the immune response. Understanding processes that govern such modulation is key to tackling conditions in which the immune system is hyperactive and responds to either non-harmful or self-antigens.

The aim of my project is to investigate the effect of keratinocyte-derived exosomes on the induction of tolDC phenotype, with specific interest on the effect of allergens on those exosomes. I will also use primary keratinocytes and a standardized cell line, as well as a keratinocyte cell line (HaCaT) with a silenced filaggrin gene, serving as a model of atopic dermatitis. Methods employed to fulfil this aim involve the use of dendritic cell models: monocyte-derived dendritic cells (MDDCs), tolerogenic DCs (tolDCs) and Langerhans Cell-like cells (LCLCs), generated from human blood monocytes. Following exosome treatment, phenotypes of those cells will be investigated by looking into their expression of specific markers and cytokine secretion profile. Next, I will also investigate downstream T cell responses, especially the induction of regulatory T cell phenotype. This will incorporate detailed characteristic of those cells and functional assays (e.g. ELISpot) to address their reactivity.