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Thesis title: The role of keratinocyte-derived small extracellular vesicles in the interaction with the immune system in atopic dermatitis

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Abstract

Keratinocytes are the main cellular component of the epidermis, the outermost layer of the skin. **Atopic dermatitis (AD) is a chronic inflammatory skin disease** in which integrity of the epidermis is compromised due to genetic factors and inflammation. As a result, pathogens, allergens, UV radiation and chemicals which come into contact with the epidermis penetrate into deeper layers of the skin triggering immune responses and causing tissue damage. Type 2 immune response predominates in AD but enhanced type 17 and type 22 responses also contribute to the inflammatory *milieu*. **Keratinocytes regulate the activity of immune cells in AD** and promote skin inflammation. **The skin of AD patients is prone to colonization by pathogens** such as *Staphylococcus aureus* (*S. aureus*) or *Candida albicans* (*C. albicans*) which exacerbate skin inflammation. *FLG* encodes profilaggrin which is expressed almost exclusively by epidermal keratinocytes. This protein is crucial for keratinocyte differentiation and maintenance of epidermal integrity. Additionally, mounting evidence suggests the involvement of **profilaggin in regulation of immune responses.** Loss-of-function (LoF) **mutations in the *FLG* gene are the greatest genetic risk factor for AD**. Such mutations also predispose AD patients to **'allergic march'** which is the appearance of additional allergic manifestations later in life; these can affect tissues devoid of *FLG* expression and occur even after the resolution of AD symptoms.

Exosomes are small lipid bilayer-enclosed vesicles produced by all nucleated cells; these vesicles mediate short- and long-distance communication between cells and tissues. Exosomes can be isolated from conditioned cell culture media or body fluids and are contained in the fraction of **small extracellular vesicles (sEVs)**. Extensive evidence shows the involvement of sEVs derived from different cells/tissues in immune responses but **the role of keratinocyte-derived sEVs (KC_{sEVs}) in AD is unknown**.

Research presented in this thesis aimed to elucidate whether **sEVs secreted by keratinocytes subjected to AD-relevant conditions carry out immune-related functions**; cultured human primary keratinocytes, human immortalized keratinocyte cell lines and human blood plasma were used as sources of sEVs.

First part of the work showed that exposure of keratinocytes to AD *milieu* and *C. albicans* increased interaction between KC_{sEVs} and dendritic cells; this was due to an enrichment of the KC_{sEV} surface in certain glycans, including those containing sialic acids. Receptor blocking experiments revealed that sialic acid-binding immune inhibitory Siglec-7 and -9 receptors were involved in the interaction between KC_{sEVs} and antigen presenting cells (APCs). ST6 β-galactoside α-2,6-sialyltransferase 1 (ST6GAL1) and core 1 β,3-galactosyltransferase 1 (C1GALT1) were upregulated by keratinocytes exposed to AD *milieu.* ST6GAL1 was also upregulated in the epidermis of AD patients. Hence, both enzymes may contribute to the remodeling of the KC_{sEV} surface glycosylation pattern upon exposure of keratinocytes to AD cytokines and *C. albicans*.

The next part of research demonstrated that KC_{sEVs} can be a source of lipid CD1a ligands which modulate CD1a-specific T cell responses; however, the activity of phospholipase A2 was required to liberate CD1a ligands from sEV membranes. *FLG* knockdown in keratinocytes (shFLG) resulted in the altered capacity of KC_{sEVs} to modulate lipid antigen-driven CD1a-specific T cell responses; specifically, type 2 response was enhanced while type 1 response was reduced. This was a consequence of a decreased abundance of stimulatory and enrichment in inhibitory lipid CD1a ligands in the membranes of sEVs produced by shFLG keratinocytes. The differences in the sEV lipidome resulted from downregulation of enzymes involved in lipid metabolism
in shFLG keratinocytes; one of these enzymes, long-chain-fatty-acid-CoA ligase 3 (ACSL3) is known incorporate long-chain polyunsaturated fatty acids into phospholipids of biological membranes. Additionally, downregulation of not only other isoforms of the ACSL enzyme but also the elongation of very long chain fatty acids enzyme (ELOVL) family was observed in AD skin. The role of ELOVL enzymes is to elongate fatty acid chains.

Finally, the last part of work documents the presence of profilaggrin-related cargo in sEVs produced by cultured keratinocytes. Additionally, such cargo was detected in sEVs derived from blood plasma of healthy individuals and AD patients. Moreover, exposure of keratinocytes to *S. aureus* enhanced the loading of profilaggrin-related products into KC_{sEVs}. The mechanism

of this involved Toll-like receptor 2 (TLR2), which is known to recognize *S. aureus* in keratinocytes. *S. aureus* also dysregulated the production of sEVs by keratinocytes; a pronounced increase in the secretion sEVs of exosomal characteristics and small microvesicles (sMVs) by those cells was observed.

Taken together, the results presented in the thesis suggest an important role for KC_{sEVs} in immune response in AD. The keratinocyte sEV system can be hijacked by pathogens as their evasion strategy; *C. albicans* promotes interaction of sEVs with immune inhibitory Siglec receptors on APCs which may impede pathogen clearance, while *S. aureus* seems to exploit the sEV-mediated removal of profilaggrin and its breakdown products to avoid their antimicrobial properties. Additionally, such enhanced removal profilaggrin-related products results in further decrease of its level in the epidermis, contributing to the barrier defect. Finally, KC_{sEVs} produced on a filaggrin-insufficiency background exacerbate type 2 inflammation in the AD skin, and potentially also in the other tissues to which they could be delivered by circulation. All these identified mechanisms may intensify inflammation in AD skin by lowering barrier quality and contribution to the atopic *milieu,* and may potentially affect other tissues and organs, with relevance to the 'allergic march'.