Keratinocyte-derived exosomes as an important factor in allergy to environmental allergens

Agata Łuczak, Laboratory of Physical Biochemistry, Intercollegiate Faculty of Biotechnology UG & MUG

Supervisor: dr hab. Danuta Gutowska-Owsiak

Exosomes are nanosized extracellular vesicles (EV) secreted by various types of cells, carrying nucleic acid, protein and lipid cargo. Recently, they have emerged as an important factor in intercellular communication in numerous physiological and pathological processes, including immune regulation. Nevertheless, data concerning their role in allergy induction are scarce.

Human epidermal keratinocytes are an important element of the innate immunity, as they create the barrier to external environment, i.e. epidermis. Thereby, they are the first to meet environmental allergens and, in the individuals suffering from atopic dermatitis or other allergic skin disease, to contribute to clinical symptoms. Epidermal keratinocytes are known to recruit and activate immune cells by chemokine attraction and cytokines, respectively. However, given that epidermal keratinocytes also produce exosomes, it is possible that these vesicles can also convey messages between keratinocytes and the immune system. Thus, we aimed to investigate their role in induction of allergy to environmental allergens.

In the study we utilised immortalized human keratinocyte cell line HaCaT, normal human epidermal keratinocytes (NHEKs) and peripheral blood mononuclear cells (PBMCs). We used 9 different environmental allergen extracts (standard allergy skin prick test solutions of Aspergillus, house dust mite, hen's egg, cow's milk, peanut, grass, weeds, birch and trees) to stimulate keratinocytes. Exosomes and other EV fractions, i.e. apoptotic bodies (APs) or microvesicles (MVs) were collected from cell culture conditioned media using differential ultracentrifugation protocol. Allergen-dependent changes in the abundance of EV-related markers (alpha-actinin-4, syntenin, stratifin, CD9 and CD63) and immunologically-relevant proteins (HLA-ABC, HLA-DR, CD1a, CD1d, CD40, CD54, CD80 and CD86) were determined by Western blot approach. Allergen extract stimulation of keratinocytes induced changes in EV compartment. Changes in the content of specific markers were observed in exosomes but not in other EV fractions. The strongest effects were observed for house dust mite, egg, milk, peanut and weeds, and those allergens were chosen for further work. In preparation for further experiments, alloreactivity of PBMCs from several healthy donors upon exposure to exosomes from

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non-stimulated HaCaTs (i.e. HLA-unmatched to PBMCs) was tested by IFNγ ELISpot assay; no changes in IFNγ secretion were observed.

Exosomes were the only extracellular vesicles showing significant change upon exposure of keratinocytes to allergens. This suggests that exosome-driven intercellular communication in allergy may be a very specific mechanism. Further work is required to determine the exact role of exosomes derived from antigen-stimulated keratinocytes in induction of allergy to environmental allergens.

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