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Institutet**

Institutionen för medicin, Solna

Exosomes – the future of vaccination?

AKADEMISK AVHANDLING

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av

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ABSTRACT

Exosomes are small membrane vesicles that are secreted by cells as means of intercellular communication. They are typically between 50 and 100 nm in diameter and originate from the endosomal compartment of cells. Exosomes have been considered a potential novel cell-free therapeutic agent since exosomes are capable of antigen presentation. Indeed, exosomes from dendritic cells can activate the innate and adaptive immune systems, can establish protective immunity in various models of infectious diseases and can eradicate established tumors in mice. However, using exosomes in the clinic has proven difficult and need optimization to induce a sufficient immune response. Research now focuses on a) using exosomes as biomarkers and diagnostic tools for neoplastic diseases and b) understanding the exosomal immune response and finding ways to increase the immunogenicity of exosomes.

This thesis aimed at 1) clarifying mechanisms important for the exosomal immune response, 2) identifying new ways to increase immunogenicity of exosomes, and 3) studying the relevance of exosomes in a human inflammatory disease, atopic eczema (AE). We report that exosomes from murine bone marrow-derived dendritic cells (DCs) can induce CD4⁺ T cell responses in a B cell-dependent manner. By comparing exosomes loaded with the whole ovalbumin (OVA) protein to exosomes loaded with the dominant CD4⁺ T cell epitope we found that only whole OVA-loaded exosomes could induce memory T and B cell responses *in vivo*. Interestingly, T cell activation was absent in Bruton kinase knockout (btk^{-/-}) mice lacking a functional B cell compartment. Further, we found that bone marrow DC-derived exosomes express CD1d and can activate natural killer T cells (NKT cells) *in vitro* and *in vivo*. Activation of NKT cells subsequently amplified innate NK cell and $\gamma\delta$ T cell responses as well as OVA-specific CD4⁺ T cell, CD8⁺ T cell and B cell responses. Our data suggest that exosome-induced antibody production is linked to subsequent activation of T follicular helper cells, germinal center B cells and plasma cells. In a third study, using exosomes from human monocyte-derived DCs, macrophages and plasma, we discovered a novel inflammatory property of exosomes. We found that exosomes contain enzymes of the leukotriene pathway and that they could produce high amounts of leukotriene B4 and C4 when incubated with the intermediate leukotriene A4. Exosomes could also induce granulocyte migration, which increased when incubated with the substrate AA. Finally, we found that the commensal yeast *Malassezia sympodialis* secreted nanovesicles that carry *M. sympodialis* allergens and induced significantly higher IL-4 responses in peripheral blood mononuclear cells (PBMC) of AE patients sensitized to the yeast than in PBMC of healthy controls (HC). Nanovesicles induced TNF- α production in PBMC of both groups. Further, we find that exosomes from monocyte-derived DCs, co-cultured with *M. sympodialis* induce significantly higher IL-4 and TNF- α responses than exosomes from unstimulated DCs in PBMC of both AE patients and HC. This suggests a role for nanovesicles in the allergic immune response.

In summary, we have identified three new pathways, which might be exploited to induce more potent immune responses to exosomes. Including B cell epitopes and CD1d ligands as well as exploiting the chemoattractive capacity of exosomes when designing future exosomal vaccines might increase the efficacy in a clinical setting. The finding that immunogenic nanovesicles are produced by *M. sympodialis* highlights novel host microbe interactions in AE and emphasizes the immunostimulatory potential of exosomes also in humans.