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INTRODUCTION

Putting EV into context: contextual factors influencing immune-related functions of extracellular vesicles (EV)

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Intercellular communication by cells of the immune system plays an important role in the induction and coordination of immune responses. Miscommunication or lack of communication between immune cells can lead to immune-related pathologies. Cells can employ several molecular mechanisms for communication with other cells. In the last decade, we have seen an enormous rise in interest in extracellular vesicles (EV) as means of intercellular communication. EV are small lipid bilayer-enclosed vesicles that are actively released by cells. An exponentially growing number of publications show that release and uptake of EV is a highly conserved means by which cells in many different organisms communicate [1, 2]. EV are present in in vitro (primary) cell cultures and in a multitude of body fluids. The 50–300-nm-sized EV contain lipids (membrane-bound), proteins, and nucleic acids. In a large variety of in vitro settings, it was demonstrated that transfer of EV can induce signaling and alter the behavior of target cells. A limited number of studies have also indicated functional effects of EV released in vivo [3].

The role of EV in the immune system is very complex [4]. Various different immune cell types can each release EV that differ in molecular composition and function. There is also strong evidence that even EV populations released by one cell type are highly heterogeneous [5], for example because they are formed via different EV biogenesis routes. The heterogeneity of EV in the immune system is increased even further because stimulation and differentiation signals imposed on cells induce changes in the number and molecular composition of released EV. Since immune cells routinely interrogate the environment and respond to cues related to danger, infection, or abnormal cell death, this will continuously affect the pool of released EV. Multiple lines of evidence indicate that EV released by immune cells can influence the activation, proliferation, and cytokine/chemokine release of other immune cells [6]. EV released by non-immune cell types, such as tumor cells and virus-infected cells, can also affect the function of immune cells [7, 8].

Although currently available data substantiate the idea that EV are important players in the immune system, we must face the fact that there are still large gaps in our understanding of how EV contribute to immune-related processes. An emerging theme within EV research is the context-dependency of EV function. This means that environmental and situational conditions during EV-target cell encounter can influence the functional effects of EV. The reviews in this issue of *Seminars in Immunopathology* provide examples of how EV-related functions change in situations where the immune system is challenged, e.g., during pregnancy, organ transplantation, inflammation, cancer, and host-pathogen interactions.

The immune system plays an imminent role at the beginning of life. Each stage of pregnancy requires different actions from the immune system, varying from pro-inflammatory reactions during implantation to immune suppression which ensures maternal-fetal tolerance. Various lines of evidence indicate that EV play a bi-directional role in communication between maternal and fetal cells in the placenta in order to orchestrate these immune-regulatory events. The review by Carlos Salomon and colleagues [9] provides a comprehensive overview of which immune-relevant molecules are found on different EV populations released by trophoblasts and how these could regulate the function of T cells and innate immune cells. The placenta-derived EV are thought to play an important role in the development and maintenance of successful pregnancies. This is substantiated by data indicating that the concentration and bioactivity of EV change in diverse pregnancy complications such as preeclampsia, gestational diabetes, and pre-term birth. The authors discuss the possible relationship between pregnancy disorders, exaggerated

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inflammatory responses, and the quantity or type of released EV. This also elicits thoughts about the diagnostic and therapeutic utility of pregnancy-associated EV.

The review by Tom Groot Kormelink and colleagues [10] also addresses the role of EV in cross-talk between cells of the immune system. Although most work on the immune-related functions of EV is focused on the adaptive immune responses, this review summarizes interesting findings on EV released by innate immune cells. It is well-established that the innate immune system plays a crucial role in the initiation and shaping of adaptive immune responses and that cellular interactions between the two arms of the immune system help to combat cancer and bacterial and viral infections. EV released by innate cells are thought to play an important role in this process. A comprehensive overview is provided of the phenotype and function of EV released by mast cells, neutrophils, and macrophages. These EV contain a wide range of different components, including microbial antigens, enzymes (e.g., lipases, elastase), cytokines and chemokines, lytic molecules, and immune-modulatory proteins. Some of these EV-associated molecules were proven to modulate adaptive immune responses, while for other molecules their contribution to EV-mediated effects needs to be confirmed.

A few of the mechanisms that drive the sorting of cytoplasmic or membrane-associated proteins into EV have been resolved [11]. However, the review by Edit Buzas and colleagues illustrates that EV should not only be seen as "pure" membrane vesicles but also as structures that can be modified by the extracellular milieu [12]. There is accumulating evidence that EV can bind several types of molecules from the external milieu, such as enzymes, ECM proteins, and antibodies, together referred to as the "interactome" of EV. The authors explain that these external molecules can either associate to EV in the extracellular milieu or bind to EV during endocytosis and re-secretion. These modifications not only affect the isolation and characterization of EV but can also determine their target cell specificity and function. This interactome of EV has been implicated in both homeostasis and in immune-related diseases such as cancer and autoimmune diseases.

The role of the extracellular milieu in steering the function of EV is further addressed in the review by Michael Graner [13], which advocates a role for purinergic receptors and their ligands (extracellular adenine nucleotides/nucleosides) in EV immunobiology. The concentration of nucleotides/nucleosides (ATP, and breakdown products such as ADP, AMP, and ADO) in local tissue environments can be highly variable. Graner points out an intricate relationship between ATP levels, EV release, and cell/EV-associated purinergic receptors on one side and immune regulation on the other. Stimulation of purinergic receptors leads to the release of EV that contain pro-inflammatory cytokines and ATP. Triggering of purinergic receptors on the surface of EV can lead to leakage of ATP from EV, causing ATP concentrations in the local environment to increase. However, particular types of EV, e.g., those derived from certain tumor cell types, contain exonucleases CD39 and CD73 that convert ATP to ADO. The latter molecule suppresses T cells and NK cells and promotes regulatory T cell function. The purinergic system is therefore thought to form part of the context in which the immune system can encounter the EV. The receptor-ligand interactions within this system may regulate whether recipient cells react to EV encounter by immune activating or immune suppressing activities.

This special issue also contains three reviews surveying the role of immune-related EV in different clinical settings, namely transplantation, virus infection, and cancer. Adrian Morelli and colleagues contributed a comprehensive review on the role of EV in "cross-dressing" during organ transplantation [14]. Cross-dressing is a process in which cells of the immune system receive major histocompatibility complex (MHC) class-I and class-II molecules loaded with antigenic peptides from other cells. This allows spreading of several types of endogenous and foreign antigens. EV-mediated transfer of pre-formed peptide-MHC complexes to antigen-presenting cells can have several implications for the ensuing immune response. Apart from a role of cross-dressing in immune homeostasis during steady-state conditions, this process can also control allo-reactive T cell responses during organ transplantation. The authors highlight that functional implications of intercellular transfer of pre-formed peptide-MHC complexes via EV depend on several factors, including the type of antigen-presenting target cell, the fate of the EV after uptake, and environmental conditions. Besides a role for cross-dressing during organ transplantation, antigen-presenting cells can also acquire peptide-MHC-I from tumor or infected cells and present these to CD8 T cells. This implies a potential role of EV-mediated MHC cross-dressing in infections and cancer.

The role of EV during virus infections is further highlighted in a review written with colleagues of my own research group [15]. Numerous studies have demonstrated that during infection with enveloped viruses, EV can transport viral subunits to other cells and herewith affect the antiviral response. Less is known about how naked virus infections affect EV release. Although naked viruses generally spread by inducing cell lysis, recent studies indicate that in the pre-lytic stage of infection, complete virions can be packed in EV and released by the infected cell [16]. Via such EV, viruses can spread and act as invisibility cloaks to evade detection by the immune system. However, uptake of virus-containing EV by immune cells has also been shown to trigger antiviral responses. In our review, we underline that changes in cell physiology induced by virus infections can cause temporal differences in EV release and EV composition over the course of infection. Combined with the fact that EV may be formed by different biogenesis routes operating in parallel, this may cause EV populations released by infected cells to be highly

heterogeneous in composition and function. Distinct pro-viral or pro-host functions may therefore be ascribed to different EV subpopulations. It is essential to dissect this heterogeneity in EV populations to further delineate the relevance of EV for viral life cycles and outcomes of viral infections.

Finally, Michael Graner and colleagues contributed a second review, dealing with the range of effects that tumor-derived exosomes and other EV (TEX) can have on the immune system [17]. Most of the TEX-induced effects lead to suppression of the immune response in order to create a favorable microenvironment that supports tumor growth. The authors give a comprehensive overview of which immune cells are affected by TEX and the variety of different molecular mechanisms underlying these effects, including triggering of inhibitory receptors, downregulation of activating receptors, and regulation of gene expression via transferred miRNAs. A comprehensive overview is provided of currently known miRNAs in TEX that affect the function of T cells, NK cells, and monocytes. Another important point addressed by the authors is the fact that functional effects of TEX may extend beyond the immune cells that bound or engulfed the TEX. Due to the complexity of immune cell interactions, there may be secondary effects imposed by TEX-modified immune cells on other immune cells. Moreover, the context in which the immune system encounters TEX, e.g., the activation status of immune cells and the cytokine milieu, influences the function of TEX.

Several of the reviews in this special issue also address technical challenges encountered in EV research. In this relatively young field, the fundamental knowledge on the formation and structure of EV is not yet up to the level that solid guidelines can be set up on how to isolate and characterize EV. Instead, the International Society for Extracellular Vesicles provides regularly updated recommendations for studying EV [18, 19]. Yet, a large range of different techniques is being used in the field to isolate EV, remove contaminating non-EV structures, and assess their function. Apart from pointing out that biases can be introduced by different EV isolation methods, the reviews in this issue also underline that contrasting data on the function of immune-related EV are likely caused by using EV-producing and EV-recipient cells that differ in activation status and by variability in culturing conditions and timing of experiments. It is therefore crucial to acquire more knowledge on how technical and conditional parameters affect the type, purity, and function of isolated EV. Initiatives have also been taken to improve reporting of methodological details in scientific publications in order to enhance the reproducibility of EV studies [20].

Overall, the reviews in this special issue highlight a number of contextual factors that influence immune-related functions of EV. First, external signaling molecules and cellular interactions influence the number and composition of EV released by producer cells. Second, molecules that can bind to EV once they have been released in the interstitial fluid or circulation affect the targeting and functional properties of EV. Lastly, the environmental conditions in which recipient cells encounter EV determine the efficiency of EV uptake, the subcellular fate of EV, and how molecular components of EV eventually trigger changes in target cell behavior. The exchange of EV by immune cells thereby provides a complex, but versatile and adaptable means by which cells can instruct or alarm neighboring cells. Putting EV into the context of immune cell interactomes and environmental conditions is essential to further unravel their role in immune pathologies and their potential use as therapeutic target or diagnostic biomarker.

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