Characterizing the contribution of inflammasome-derived exosomes in the activation of the immune response

Miguel Abal

Translational Medical Oncology, Health Research Institute of Santiago (IDIS), University Hospital of Santiago (SERGAS), Trav. Choupana s/n 15706 Santiago de Compostela, Spain

Correspondence to: Miguel Abal. Translational Medical Oncology (IDIS/SERGAS), Trav. Choupana s/n 15706, Santiago de Compostela, Spain. Email: miguel.abal.posada@sergas.es.

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The interaction of cells with the environment is well known and documented in different processes associated with the fate of the diseases. How do different cells from different organs with different and complex environments communicate in a global and integrative manner is a question that researchers from complementary scientific areas are trying to address over the past decades. Extracellular vesicles (EV), small vesicles secreted by most cell types, participate in intercellular communication allowing exchange of proteins, lipids and nucleic acids between the EV-producing and target cells. Among them, exosomes as vesicles with diameters of 30–150 nm intervene in the transfer of proteins, mRNAs, and miRNAs to recipient cells to mediate many biological processes. The impact of this type of intercellular communication and its relevance at the clinical setting is being recognized by the scientific community, and the role of exosomes as mediators of this interaction is becoming a new and exciting field of research with promise to address relevant clinical questions in the next future.

A recent paper approaches the study of exosomes as mediators of the inflammasome, complex multimeric protein complexes formed during innate immune response (1). The intercellular transfer of pathogen- or host-derived RNA, DNA and proteins from infected cells to neighbor cells impacts on host innate immunity (2). Strong evidence also indicates that exosomes-delivered microRNAs undergo a functional transfer between immune cells and constitute a mechanism of regulating the inflammatory response (3). But it is now clear that not only immune cells but probably all cell types are able to secrete exosomes: their range of possible functions expands well beyond immunology to neurobiology, stem cell and tumor biology, and their use in clinical applications as biomarkers or as therapeutic tools is an extensive area of research (4,5).

An effective innate immune response relies on the detection of pathogen associated molecular patterns (PAMPs) by various host pattern recognition receptors (PRRs) that result in the production of pro-inflammatory cytokines and chemokines. Exosomes have been proposed to mediate one of the mechanisms involved in the stimulation of P2X purinoceptor 7 receptor (P2X7R) that rapidly triggers a key step of the inflammatory response: the induction of NLRP3/caspase-1 inflammasome signalling complexes that drive the proteolytic maturation and secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18) (6). ATP stimulation through a P2X7R-dependent pathway activates robust vesicle-mediated unconventional protein secretion, including exosome release and membrane shedding, and drives NLRP3 inflammasome activation in a calpain activity dependent manner (7). Also related, central nervous system trauma induces inflammasome activation and secretion of exosomes containing inflammasome protein cargo into

cerebral spinal fluid that fuse with target cells to activate the innate immune response in peripheral tissues (8).

In this new work, the group of Dr. Wang describes a proteomic approach to the inflammasome signaling and exosome secretion by challenging murine macrophages with the agonist of NLRP3 inflammasome signaling LPS/nigericin. Two main issues systematically impact on this type of studies: first, the design of the model system that must address a complex and dynamic scenario involving different cellular systems and a highly interactive environment, and second, an appropriate methodological approach for the purification and characterization of the EV and exosomes. This study includes the generation of bone-marrow-derived macrophages (BMDM) from 8-week-old C57BL/6 mice cultured for 7 days, with 98% isolation efficiency as evaluated by $CD11b^{+}/F4/80^{+}$. Activation of NLRP3 inflammasome signaling pathway has been conducted in two-step stimulation, first by treatment of BMDMs with endotoxin for 6 h, and second challenged with nigericin for 30 min; BMDMs treated with endotoxin and mock were used as controls. In addition to the characterization of the proteome from inflammasome-associated exosomes, their functional characterization includes microscopy visualization of fluorescently-labelled exosomes internalization by BMDMs, the triggering of pyroptosis, a Caspase-1 dependent pro-inflammatory form of programmed cell death, and the secretion of cytokines.

Exosomes from culture supernatants [fetal bovine serum (FBS) used in culture was previously depleted of endogenous exosomes by overnight centrifugation] were isolated by differential centrifugation. Proteomic analysis was performed by LC-MS/MS, comparing three biological replicates from each condition. The presence of exosomal proteins confirmed the origin of exosomes, with a 50% overlap of the total identified proteins from the different stimulus and a 20% of specific proteins associated with the inflammasome activation by LPS/nigericin, mainly up-regulated. These proteins were involved in immune system process, innate response and inflammatory response; together with proteins of the inflammasome signaling pathway, toll-like receptors and members of the tumor necrosis factor (TNF) and nuclear factor-κB (NF-κB) families were highly enriched in exosomes with treatment of LPS/nigericin, further reinforcing the relationship between inflammasome-derived exosomes to the immune response and infection. In addition to the proteomic characterization, inflammasome-derived exosomes were found to be preferentially uptaken by macrophages leading to the up-regulation of NLRP3 and IL-1β, processing of Caspase-1 and triggering of pyroptosis. Finally, the analysis of secreted cytokines upon treatment with inflammasome-derived exosomes further confirmed the activation of the NF-kB signaling pathway.

This work demonstrates the ability of exosomes derived from the inflammasome to enter macrophages and activate the immune response, amplifying the inflammatory signaling in neighbor cells. The next challenge is to add complexity to the model systems simulating these eventual therapeutic opportunities; exosomes from different cell type origins in a more interactive 3-dimensional co-culture environment and submitted to dynamic processes would ideally result in a more clinically relevant conclusion. Also, the transference of other cargo into exosomes mediating NLRP3-inflammasome activation like microRNA (9,10), or the involvement of other EV during acquired and innate immunity (11), would provide a more complex and complete overview of the interaction of cells with the environment. Finally, the potential modulation of this process may serve to potentiate the immune response in cancer immunotherapy or in the control of autoimmune diseases; innovative approaches like DNA vaccines encoding extracellular vesicle-associated antigens represent promising immunotherapy tools for different diseases (12).

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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