Editorial Commentary

Why should the molecular characterization of inflammasome-induced exosomal cargo be done?

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Exosomes (EXSs) are nano-sized and circulating membrane-bound extracellular vesicles (EVs) which protect the stability of their cargo, including messenger ribonucleic acid (mRNA), microRNA (miRNA) and other proteins from degradation. Upon taken up by recipient cells, they deliver their cargo molecules to the target cells. When inflammatory stimuli activate innate immune signaling receptors, cargo molecules modify the molecules of recipient cell. Because molecules carried with EXS-cargo are thought to be biomarkers, the role of EXSs is emerging as an important new area of biomedical research.

In addition to inflammatory mediators, EXSs, which are secreted by macrophages play pivotal roles in intercellular communications and multi biological functions (1). The immune response-related proteins and signaling pathways are specifically enriched in inflammasome-derived EXSs. This type of EXSs directly activates nuclear factor kappa B (NF-κB) signaling pathway and expresses pro-interleukin 1-beta (pro-IL-1β) and pro-IL-18 via its transcriptional upregulation (2).

EXSs as potential carriers for targeted gene, are thought to promote inflammation via releasing of inflammasome components during the inflammatory response. Of note, there is a need for further studies to elucidate the role of EXSs by using improved detection and characterization methods (3). Indeed, inflammasomes are critical for both local and systemic inflammation. Canonical type NOD-like receptor protein 3 (NLRP3) inflammasome is known to sense multiple microbial and endogenous danger signals, and its activation results in caspase-1, IL-1β and IL-18, dependent processes. However, non-canonical, caspase-4/5-dependent inflammasomes bind to intracellular lipopolysaccharides (LPS), and directly regulate pyroptosis (4,5). Contrarily, Budden *et al.* have proposed that the pathways eliciting caspase 1-driven cell death strongly induce EXS release, whereas stimuli, which do not induce pyroptosis are weaker stimuli for EXS release (6). In this context two noteworthy results are emerged in this study. EXSs released from inflammasome-activated macrophages have a specific RNA signature and contain interferon-beta (IFN-β). IFN-β is responsible for the induction of IFN-stimulated genes (ISGs) in EXS recipient cells, independently of NLRP3 expression. Thereby, this cargo induces an IFN signature in bystander cells and leads to silencing of NLRP3 inflammasome response (6). In this situation, IFN-β inhibits NLRP3 inflammasomes in a signal transducer and activator of transcription 1 (STAT1)-dependent manner, while decreases the amounts of pro-IL-1β in a STAT3-dependent manner (7). In case of toll-like receptor (TLR) activation, Toll/IL-1 receptor homology (TIR)-domain-containing adaptor protein-inducing IFN-β (TRIF) interacts with receptor-interacting protein 1 (RIP1), which is responsible for the activation of NF-κB. Thus, stimulation with TLR ligands induces type I IFN production in addition to proinflammatory signals via TRIF-dependent pathway (8). However, activation of inflammasome controls the post-translational proteolytic activity of IL-1β, IL-18, pore-forming gasdermins and caspase-1. Pyroptosis commonly occurs with the activation of the caspase-1-dependent canonical pathway and the

caspase-4/5/11-dependent non-canonical pathway (9). In the first phase of inflammasome activation, LPS binds to TLR4. When macrophages are primed by LPS, activation of NF-κB signaling promotes expression of inflammasome-related molecules, including pro-IL-1β and NLRP3 (10). Budden *et al*. showed that EXS release upon NLRP3 activation is temporally correlated with IL-1β release and is an NLRP3-, caspase 1-, and gasdermin D-dependent event. In this study, it is noted that the transcriptional cargo of EXSs is released by human macrophages upon stimulation with inflammasome or TLR stimuli. However, RNA content of EXS, which is induced by different NLRP3 activators defined as EXS-associated NLRP3 signature is different from the RNA content of TLR-induced EXSs (6).

To express inflammasome-related molecules, following the degradation of inhibitor kappa B (IκB), NF-κB undergoes nuclear translocation and binds to the promoter regions of its downstream target genes (11). Wang *et al*. showed that LPS-induced expression of NLRP3 and pro-IL-1β, is dependent on NF-κB signaling. Thus, in connection with the transcriptional state, EXSs may inhibit IL-1β production and pyroptosis in macrophages by suppressing the NF-κB signaling pathway in the LPS-priming phase, but independently of the NLRP3 inflammasome activator, nigericin-triggering phase (12).

As mentioned above, EXSs can carry functional proteins and RNAs related to many diseases. Since EXSs represent cell-to-cell communication mediators (13), and their contents are protected from degradation, they are thought to be useful biomarkers considering the inflammasome activation (14). Furthermore, these functional RNAs can be transported from an EXS donor cell to recipient cells, and enable transcriptional changes (15). Although there are extensive studies on biological functions of EXS in inflammatory diseases, mechanistic explanations of how the inflammasome activity regulates secretion of EXS, and how their cargo modulates the biological properties of inflammatory cells are unclear (4). Thus, NLRP3 inflammasome, which is known to be released from damaged cells may contribute to the development of acute graft-versus-host disease via IL-1β-dependent mechanism. Increased levels of active caspase-1 and IL-1β are found in circulating leukocytes and lesions of transplant patients (16). Moreover, nucleic acids containing EXSs—especially miRNAs and long noncoding RNAs (lncRNAs) have been shown to mainly influence insulin signals in target tissues, affecting cell viability, and modulating inflammatory pancreatic cells. In this context, it is thought that the EXS miRNAs can be used as reliable biomarkers in diabetes (17). Additionally, in diabetes mellitus, it is claimed that the EXSs cargo may signal to transform the endothelial cells from normal phenotype into a diabetic phenotype. However, it is still unclear whether EXS is a precise marker for the detection of obesity related diabetes mellitus (18). Recently, characterization of the circulating exosomal proteins in SARS-CoV-2 infection has revealed the role of macrophage activation syndrome as the main driver of hyperinflammatory response in the mechanisms associated with tissue damage and multiple organ dysfunctions in coronavirus disease-19 (COVID-19) patients (19). On the other hand, tumor-derived EXSs convert macrophages to tumor-promoting type via regulating proteasomal degradation, and activate NLRP3 inflammasome signaling pathway, which promotes lung cancer progression by IL-1β secretion (20). However, the potential participation of macrophage derived EXSs to heterotypic cell communication in tumors has been poorly studied.

In brief, EXSs as therapeutic tools for potential use in medicine, have properties for allowing the identification of new biomarkers (21). Actually, atheroprotective Kruppel-like factor regulates inflammation-associated miRNA-155 expression in human endothelial cells. When miRNA-155 plus ox-LDL-induced endothelial cells derived EXSs are transferred to human monocytic THP1 cells, it enhances the shifting of the monocytes/macrophages balance from anti-inflammatory M2 macrophages towards proinflammatory M1 macrophages. This alteration exerts a regulatory effect on NLRP3 inflammasome activation in macrophages. Consequently, EXSs from Kruppel-like factor 2-expressing endothelial cells suppress monocyte activation and diminish proinflammatory response (22,23). Similarly, the proinflammatory M1-like-type macrophages release proinflammatory cargo carrier EXSs after myocardial infarction, thereby myocardial injury is accelerated. These EXSs also contain proinflammatory miRNAs, which are transferred to endothelial cells, leading to the inhibition of angiogenesis and cardiac function by downregulating its novel target genes. Although this mechanism provides a novel insight into the therapeutic approach, how it works still could not be elucidated (24). It is observed that tumor-associated macrophage (TAM)- EXSs comprises signature of 62 proteins. While TAMs are largely immunosuppressive, their EXSs may also have the potential to stimulate, rather than limit, anti-tumor immunity. Therefore, the two faces of TAM-EXS signature can influence the tumor immunity in two opposite directions (25,26).

These studies suggest that EXS secretion constitutes an essential part of the NLRP3 inflammasome-mediated immune response. Although, it was found that the inflammasomes trigger EXS secretion, the content of exosomal cargo and its case specific properties has remained uncharacterized. In this respect, Budden *et al*. have characterized the transcriptional cargo of EXSs released by human macrophages upon stimulation with multiple inflammatory triggers, including inflammasome and TLR stimuli (6).

Budden *et al*. firstly, showed that LPS plus nigericin increased inflammasome responses when transferred to pre-primed macrophages. In contrast, subsequent inflammasome activation was inhibited when LPS plus nigericin or LPS plus R837 (NLRP3 activators) were transferred to unprimed primary macrophages. These data demonstrate that inflammasome-triggered EXSs can modulate the impact of $EXSs_{NLRP3}$ on the transcriptome of recipient cells. In other words, $EXSs_{NLRP3}$ could either increase or decrease inflammatory responses depending on the recipient cells' priming state and control the severity of inflammation (6).

Inconsistency between the content of inflammasome induced EXSs and their effects on gene expression in recipient cells constitutes a highly important finding. The characterization of EXSs content by type of EXSs and EXS inducing conditions will open a new field of research to uncover the mechanisms behind the function of the EXS.

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