Editorial

Exosomes derived from stimulated monocytes promote endothelial dysfunction and inflammation *in vitro*

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During the last few years, the scientific community interest on the role of extracellular vesicles (EVs) in physiology and pathophysiology of several human diseases has increased exponentially (1). These vesicles present the capability of transferring different kind of molecules (lipids, RNAs, DNA, protein…) between cells and may exert some effects on the cell phenotype. The content of these vesicles can vary depending on the cell type of origin (2). Although nowadays there is no consensus regarding the appropriate nomenclature, three well-known types of vesicles can be categorized on the basis of size and biogenesis: apoptotic bodies (>1 μm), microvesicles (150 nm–1 μm, budding from plasma membrane) and exosomes (30–150 nm, formed within the endosomal network and released upon fusion of multi-vesicular bodies with the plasma membrane) (1,2). Exosome is the most commonly used term to designate any type of EV; it has become a "catchword" for EV-related science. Despite the fact that the isolated fractions generally studied consist of a mixture of EVs. Unfortunately, the EVs scientific community is not able to propose a list of EV-specific markers to classify subsets of EVs (3).

Even though there are numerous methods for the isolation and purification of EVs, with ultracentrifugation considered as the "gold standard", there is not a single technique which allows the separation of the different EVs populations efficiently, that is why the International Society for Extracellular Vesicles (ISEV) recommends the use of various techniques with the goal of obtaining the purest (i.e., the most exosomes-enriched) fractions (4). Regarding the vesicles characterization, it is also recommended to combine the use of different techniques (three or more) which would allow confirming that actually, this kind of vesicle is present in the sample.

Nowadays, methods based on polymeric precipitation have become very trendy due to their easy and quick usage, but surely these are not adequate techniques for different reasons: (I) it does not avoid the co-precipitation of non-exosomal components (lipoproteins and the culture medium components) that can interfere on the biological properties of the EVs; (II) the loss of an EV-population of interest, due to the limitation of this technique to precipitate the smallest EVs (5). Culture medium components may possibly exert some kind of interaction with the target cells that could alter the biological effect associated with the studied EVs: even on commercially labeled exo-free culture media after routine quality assays, different RNAs have been detected (6,7). Henceforth the standardization of a unique EV isolation technique is necessary that could prevent the presence of non-exosomal components in EV pellets.

Exosomes vesicles have been associated with the progression of different diseases, including cardiovascular

diseases and cancer. Exosome release from tumorous cells contributes to the tumor progression facilitating the angiogenesis and metastasis (8,9). Additionally, pro-inflammatory conditions, such as atherosclerosis, promote the release of EVs derived from vascular endothelial cells, smooth muscle cells, macrophages and other immune cells that have mainly pro-inflammatory properties, which accelerates the development of vascular diseases (10). The capability of these EVs to transport and deliver biomolecules contained therein makes these nanovesicles a powerful tool for the prognosis and diagnosis of different diseases.

On the study carried out by Tang and colleagues entitled "*Monocyte exosomes induce adhesion molecules and cytokines via activation of NFκB in endothelial cells*", the authors analyzed the effects of EVs derived from stimulated monocytes with IFNα, LPS, or a combination of the two of them (I/L). The authors observed a variation of the exosomal cargo regarding the stimuli applied: when EVs from LPS or I/L stimulated monocytes were added to HUVEC cells an increase in pro-inflammatory molecules (ICAM-1, CCL-2 and IL-6) were observed when compared with IFNα stimulated monocytes and non-stimulated monocytes. Thus, the authors conclude that the EVs from LPS or I/L stimulated monocytes could be related to the formation of atherosclerotic plaques by inducing an overexpression of ICAM-1, CCL-2 and IL-6, via NFκB activation, and consequently favoring the adhesion of monocytes and their subsequent migration to the subendothelial space (11).

Gao *et al.* also demonstrated that exosomes from mature dendritic cells (stimulated with LPS) were capable of favoring endothelial inflammation and atherosclerosis via TNFα-mediated NFκB pathway in both *in vivo* and *in vitro* assays (12).

The results of this study together with the results published by Tang *et al.* seem to confirm that in the presence of certain stimuli (e.g., LPS), monocytes and dendritic cells secrete EVs that could promote endothelial inflammation and atherosclerosis progression.

The exosomal features and their potential role in inflammatory modulation suggest a possible clinical use of EVs as biomarkers or therapeutic agents. The fact that EVs have been isolated from various biological fluids (blood, urine, saliva, breast milk) (13) and the increasing knowledge of their cargo makes them useful both in prognosis and in early diagnosis of different diseases, since it is possible to identify expression patterns of different molecules such as miRNAs, specific for different types of diseases (14).

The authors of this manuscript detected differences on two miRNAs expression in exosomes from stimulated monocytes. On the one hand, an overexpression of miRNA-155, involved in inflammation and signaling via TLR-4 was identified; on the other, a downregulation of miRNA-223 was detected, which is involved mainly in cardioprotective and anti-inflammatory processes.

It is necessary to identify the pathways implicated on the exosomes mediated IL-6 induction. Most of the proinflammatory genes, including those codifying IL-6, E-selectin, ICAM-1 y VCAM-1 have been reported in the literature that may be regulated via JNK pathway (15).

Finally, the long term stimulation of immune cells by HIV seems to activate a novel mechanism in which exosomes could play a crucial role in endothelial cell communication, transferring proinflammatory factors, promoting endothelial dysfunction and inflammation; and as a consequence of all this, enhancing atheroma plaque formation and atherosclerosis development in HIV patients. However, it is essential to identify the exosomal molecules responsible for these proinflammatory and proatherosclerotic effects (miRNA, lipids or proteins) and to perform *in vivo* assays to contrast with *in vitro* studies.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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