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Extracellular mitochondria and vesicles: new mediators of endothelial inflammation

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Monocyte interaction with endothelial cells is regarded as a key step in acute and chronic vascular inflammatory diseases. For many years unravelling cell secretome composition has been an area of extensive investigation to better understand how paracrine exchange of biological information functionally impacts recipient cells. These paracrine factors include growth factors, neurotransmitters, chemokines or cytokines. Over the last two decades, extracellular vesicles have emerged as important players in conveying and disseminating biological information from activated cells to remote quiescent cells. Extracellular vesicles result either from the fusion of multivesicular bodies with cellular membrane or from the protrusion and scission of the plasma membrane, which causes the release of either small vesicles (e.g., exosomes) or large vesicles (called microvesicles; MVs) into the extracellular milieu. Due to their different subcellular origins, it is not surprising that extracellular vesicles express different subsets of proteins, lipids and nucleic acids. The heterogenous composition of extracellular vesicles determines which cell type they target and the functional consequences of their incorporation. Thus, extracellular vesicles might promote either beneficial effects, including pro-repair angiogenesis, or deleterious ones, such as pro-inflammatory responses.[1]

A large body of experimental evidence for extracellular vesicle functional roles stems from *in vitro* experiments using cell culture and conditioned medium. These findings are important initial steps to addressing the clinical relevance and therapeutic potential of extracellular vesicles in medicine. Fewer studies have examined their role *in vivo* and their contribution to the development of cardiovascular pathologies.[2,3] In this issue of *Circulation Research*, Puhm *et al.*[4] demonstrate how the monocyte secretome disseminates vascular inflammation in sepsis and identify both free and MV-associated mitochondria as key mediators.

Puhm *et al.*'s elegant study extends initial observations reporting the presence of mitochondrial proteins in monocyte MVs[5] and of interleukin-1β in MVs isolated from monocytes exposed to lipopolysaccharides (LPS).[6] Using the human monocytic cell line THP-1, the authors first compared MVs derived from normal and LPS-stimulated cells. By means of flow cytometry, quantitative PCR and Western blot analysis, they demonstrated that LPS stimulation results in the release of free mitochondria, as well as MVs enriched in mitochondrial components. Functional analyses revealed that these vesicles induced a

proinflammatory response from endothelial cells, as shown by a dose-dependent secretion of interleukin-8 and a marked increase in ICAM-1 and VCAM mRNA levels. However, this biological effect cannot be explained solely by an increase in mitochondrial components, since even high concentrations of MVs derived from unstimulated monocytes could not induce such a response from endothelial cells. Surprisingly, mitochondria isolated from LPS-stimulated monocytes presented the same capacity as MVs to activate endothelial cells. This suggests that proinflammatory elements are generated in activated monocytes and are subsequently released to the extracellular space associated with free mitochondria and with mitochondria embedded in MVs. Interestingly, the proinflammatory potential of these elements seems to depend on the mitochondrial activity of parental cells. The authors showed that the ability of monocytic MVs and mitochondria to activate endothelial cells was greatly reduced when they were isolated from parental monocytes with preserved mitochondrial membrane potential or reduced levels of reactive oxygen species. These findings imply that changes in mitochondrial activity are crucial to determining the specific proinflammatory components secreted by the monocytes. Of note, both free and MV-bound mitochondria cause TNFα and type I interferon-mediated inflammatory responses in endothelial cells. Transcriptomic profiling revealed that MVs and mitochondria isolated from LPS-activated monocytes activate tumor necrosis factor α (TNFα) and type I interferon (IFN) signalling pathways in endothelial cells, prevented respectively by a TNFα blocking antibody and an IFN decoy receptor. The authors further demonstrated that TNFα is directly associated with mitochondria released from activated monocytes and that—oxidized mitochondrial RNA activates the interferon pathway. Further studies will be necessary to determine the molecular mechanisms underlying uptake of MV-embedded mitochondria by endothelial cells. Importantly, Puhm *et al.* provide *in vivo* relevance of their *in vitro* findings in healthy volunteers exposed to low-grade endotoxemia by showing increased plasma levels of TOM22-positive vesicles, TNFα, and soluble VCAM-1. The cellular origin of circulating TOM22-positive vesicles remains unknown however and the causal effect of TOM22-positive vesicles is not demonstrated *in vivo*.

Thus, the work of Puhm et al. deepens our knowledge regarding the complexity of cell-to-cell communication by identifying mitochondria from stressed monocytes as a source of new functional mediators of endothelial inflammation in low-grade endotoxemia. This

effect, which is mediated by both free and MV-embedded mitochondria, adds to the previously shown proinflammatory effect of monocytic MVs harbouring interleukin-1β cytokines .[4,6] Surprisingly, free and MV-embedded mitochondria appear to have the same proinflammatory effect on endothelial cells.[4] Future studies will determine whether or not coating mitochondria with membrane vesicles during extracellular release protects them from triggering immune responses, as compared to free circulating mitochondria. Mitochondrial damage-associated molecular patterns have been identified in human circulating blood.[11]

Interestingly, monocytes are not the sole cell type to release free and MV-associated mitochondria as part of their secretome. Similar observations have been made for activated platelets,[7] injured hepatocytes[8,9] and mesenchymal stem cells exposed to oxidative stress.[10] Hydrolysis of extracellular mitochondria released from activated platelet by secreted phospholipase A2 generates inflammatory mediators such as lysophospholipids, fatty acids and mitochondrial DNA, which then promote endothelial inflammation.[7] Mitochondrial DNA contained in circulating hepatocyte MVs contributes to non-alcoholic steatohepatitis by activating the TLR-9 pathway.[9] Mesenchymal stem cells manage oxidative stress by expelling depolarized mitochondria in microvesicles, which are subsequently engulfed by macrophages, resulting in increased bioenergetics.[10] All together, these findings suggest that the release of free and MV-associated mitochondria in the extracellular medium may be a general response to cell activation

To conclude, the exciting results of Puhm *et al.* demonstrate the pro-inflammatory role of free or MV-associated mitochondria on endothelial cells in conditions mimicking low-grade endotoxemia. Their findings also highlight the richness of cell-to-cell communication in cardiovascular disease development. They also suggest developing more integrated approaches to understanding the role of the activated cell secretome in propagating biological signals to remote quiescent cells.

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References

1 Ridger VC, Boulanger CM, Angelillo-Scherrer A, Badimon L, Blanc-Brude O, Bochaton-Piallat M-L, Boilard E, Buzas EI, Caporali A, Dignat-George F, Evans PC, Lacroix R, Lutgens E, Ketelhuth DFJ, Nieuwland R, Toti F, Tunon J, Weber C, Hoefer IE. Microvesicles in vascular homeostasis and diseases. Thromb Haemost 2017;117:1296–1316. doi:10.1160/TH16-12-0943.

2 Boulanger CM, Loyer X, Rautou P-E, Amabile N. Extracellular vesicles in coronary artery disease. Nat Rev Cardiol 2017;14:259–272. doi:10.1038/nrcardio.2017.7.

3 Coumans FAW, Brisson AR, Buzas EI, Dignat-George F, Drees EEE, El-Andaloussi S, Emanueli C, Gasecka A, Hendrix A, Hill AF, Lacroix R, Lee Y, Leeuwen TG Van, Mackman N, Mäger I, Nolan JP, Pol E Van Der, Pegtel DM, Sahoo S, Siljander PRM, Sturk G, Wever O De, Nieuwland R. Methodological guidelines to study extracellular vesicles. Circ Res 2017;120:1632–1648. doi:10.1161/CIRCRESAHA.117.309417.

4 Puhm F, Afonyushkin T, Resch U, Obermayer G, Rohde M, Penz T, Schuster M, Wagner G, Rendeiro A, Melki I, Kaun C, Wojta J, Bock C, Jilma B, Mackman N, Boilard E, Binder C. Mitochondria are a subset of extracellular vesicles released by activated monocytes and induce type I IFN and TNF responses in endothelial cells. 2019. doi:10.1161/CIRCRESAHA.118.314601.

5 Bernimoulin M, Waters EK, Foy M, Steele BM, Sullivan M, Falet H, T WM, Bartenva N, Geng J-G, Hartwig JH, Maguire PB, Wagner DD. Differential stimulation of monocytic cells results in distinct populations of microparticles. J Thromb Haemost 2009;7:1019–1028. doi:10.1111/j.1538-7836.2009.03434.x.

6 Wang J, Williams JC, Davis BK, Jacobson K, Doerschuk CM, Ting JP, Mackman N. Monocytic microparticles activate endothelial cells in an IL-1β–dependent manner. Vasc Biol 2011;118:2366–2375. doi:10.1182/blood-2011-01-330878.

7 Boudreau LH, Duchez A-C, Cloutier N, Soulet D, Martin N, Bollinger J, Rousseau M, Naika GS, Tania L, Laflamme C, Farndale RW, Pouliot M, Hamzeh-Cognasse H, Cognasse F, Garraud O, Nigrovic PA, Guderley H, Lacroix S, Thibault L, Semple JW, Gelb MH, Boilard E. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A 2 to promote inflammation. Blood

2015;124:2173–2183. doi:10.1182/blood-2014-05-573543.

8 Cai Y, Xu M, Koritzinsky EH, Zhou Z, Wang W, Cao H, Yuen PST, Ross RA, Star RA, Liangpunsakul S, Gao B. Mitochondrial DNA–enriched microparticles promote acute-on-chronic alcoholic neutrophilia and hepatotoxicity. JCI Insight 2017;2. doi:10.1172/jci.insight.92634.

9 Garcia-Martinez I, Santoro N, Chen Y, Hoque R, Ouyang X, Caprio S, Shlomchik MJ, Coffman RL, Candia A, Mehal WZ. Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9. J Clin Invest 2016;126:859–864. doi:10.1172/JCI83885DS1.

10 Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, Stolz DB, Watkins SC, Di YP, Leikauf GD, Kolls J, Riches DWH, Deiuliis G, Kaminski N, Boregowda S V., McKenna DH, Ortiz LA. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. Nat Commun 2015;6:1–15. doi:10.1038/ncomms9472.

11 Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 2010;464:104–107. doi:10.1038/nature08780.Circulating.

Figure. Activated monocytes release free and microvesicle-embedded mitochondria which have proinflammatory effects on endothelial cells. Exposure to LPS leads to alterations in the mitochondrial activity of monocytic cells. These mitochondria accumulate damage-associated molecular patterns (DAMPs) such as tumor necrosis factor α (TNFα) as well as reactive oxygen species, which lead to oxidation of mitochondrial RNA. Activated monocytes release free dysfunctional mitochondria and microvesicles (MVs) with mitochondrial content, into the extracellular space. Upon interacting with endothelial cells, these monocytic MVs and mitochondria induce TNFα and type I interferon (IFN) signaling pathway, ultimately leading to the upregulation of inflammatory cytokines such as interleukin 8 (IL-8), and adhesion proteins like intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM).

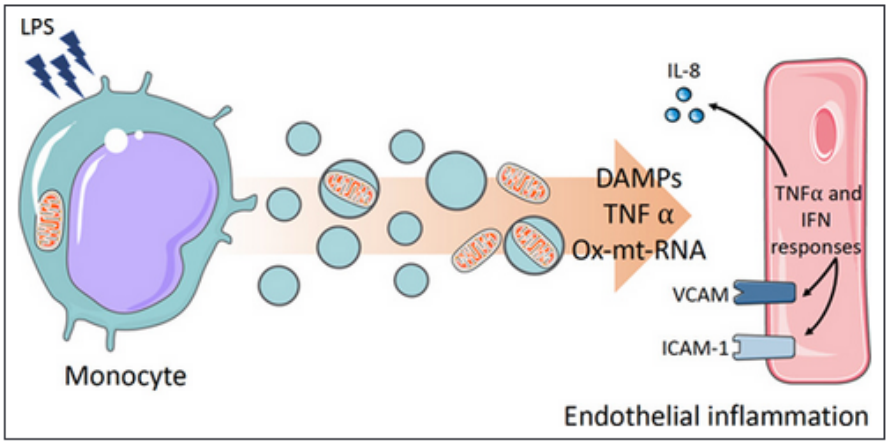

Figure. Activated monocytes release free and microvesicle-embedded mitochondria which have proinflammatory effects on endothelial cells. Exposure to lipopolysaccharides (LPS) leads to alterations in the mitochondrial activity of monocytic cells. These mitochondria accumulate damage-associated molecular patterns (DAMPs) such as TNF-α (tumor necrosis factor α) as well as reactive oxygen species, which lead to oxidation of mitochondrial RNA. Activated monocytes release free dysfunctional mitochondria and microvesicles (MVs) with mitochondrial content, into the extracellular space. On interacting with endothelial cells, these monocytic MVs and mitochondria induce TNF-α and type I IFN (interferon) signaling pathway, ultimately leading to the upregulation of inflammatory cytokines such as IL-8 (interleukin 8), and adhesion proteins like ICAM-1 (intercellular adhesion molecule 1) and VCAM (vascular cell adhesion molecule).