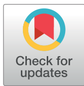

thematic review series

Thematic Review Series: Exosomes and Microvesicles: Lipids as Key Components of their Biogenesis and Functions

Extracellular vesicles and their content in bioactive lipid mediators: more than a sack of microRNA

Eric Boilard[1]

Centre de Recherche du CHU de Québec – Université Laval, Department of Infectious Diseases and Immunity, Quebec City, QC, Canada, and Canadian National Transplantation Research Program, Edmonton, AB, Canada

Abstract Extracellular vesicles (EVs), such as exosomes and microvesicles, are small membrane-bound vesicles released by cells under various conditions. In a multitude of physiological and pathological conditions, EVs contribute to intercellular communication by facilitating exchange of material between cells. Rapidly growing interest is aimed at better understanding EV function and their use as biomarkers. The vast EV cargo includes cytokines, growth factors, organelles, nucleic acids (messenger and micro RNA), and transcription factors. A large proportion of research dedicated to EVs is focused on their microRNA cargo; however, much less is known about other EV constituents, in particular, eicosanoids. These potent bioactive lipid mediators, derived from arachidonic acid, are shuttled in EVs along with the enzymes in charge of their synthesis. In the extracellular milieu, EVs also interact with secreted phospholipases to generate eicosanoids, which then regulate the transfer of cargo into a cellular recipient. Eicosanoids are useful as biomarkers and contribute to a variety of biological functions, including modulation of distal immune responses. Here, we review the reported roles of eicosanoids conveyed by EVs and describe their potential as biomarkers.—Boilard, E. **Extracellular vesicles and their content in bioactive lipid mediators: more than a sack of microRNA. *J. Lipid Res.* 2018.** 59: **2037–2046.**

Supplementary key words exosomes • microvesicles • eicosanoids • platelets • neutrophils • cancer

Extracellular vesicles (EVs) are membrane-bound vesicles that can be released from any cellular lineage, including eukaryotic, prokaryotic, and plant cells. They encompass microvesicles (also well known as microparticles or ectosomes), produced by plasma membrane outward budding and shedding; exosomes, stored in multivesicular bodies and secreted through the endosomal network; and larger vesicles known as apoptotic bodies generated during the vesiculation of apoptotic cells (**Fig. 1**) (1–4).

The liberation of EVs implicates membrane trafficking pathways, such as the endosomal sorting complex required for transport (ESCRT) system, in the budding of vesicles in the lumen of endosomes, and fusion with the plasma membrane in the case of exosome release (3, 5). The ESCRT system can also participate in the release of microvesicles (6, 7), together with scramblase and flippase activities (8, 9). The EV content may vary according to the corresponding cellular source and may differ depending on the cell activation trigger involved. Moreover, the means of release also impacts the EV cargo, and observations from numerous studies confirm that certain proteins are enriched in exosomes while others appear enriched in microvesicles. For instance, transmembrane proteins such as tetraspanins (CD9, CD63, CD81), and tumor susceptibility gene 101 and Alix, accessory molecules from the endosomal sorting complex, are mostly associated with exosomes (10). Conversely, proteins of organelle origin, such as those from the endoplasmic reticulum, Golgi, mitochondria, or nucleus, are preferentially found in microvesicles and are rarely found in exosomes (10, 11).

Extracellular vesicles imperatively comprise a lipid moiety, and their cholesterol, sphingomyelin, phosphatidylserine, and glycosphingolipid content is richer than their cellular sources (11–16). The studies that originally reported their presence in blood determined that the EV membrane could support the coagulation cascade (2, 17). Hence, a proportion of microvesicles expose phosphatidylserine at the surface, which may facilitate the deposition

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Abbreviations: AA, arachidonic acid; DC, dendritic cell; ESCRT, endosomal sorting complex required for transport; EV, extracellular vesicle; GPIb, glycoprotein Ib; 12-LO,12-lipoxygenase; LT, leukotriene; MDSC, myeloid-derived suppressor cell; miRNA, microRNA; PG, prostaglandin; PLA_2, phospholipase A_2; $sPLA_2$, secreted PLA_2.

[1] To whom correspondence should be addressed.
e-mail: eric.boilard@crchuq.ulaval.ca

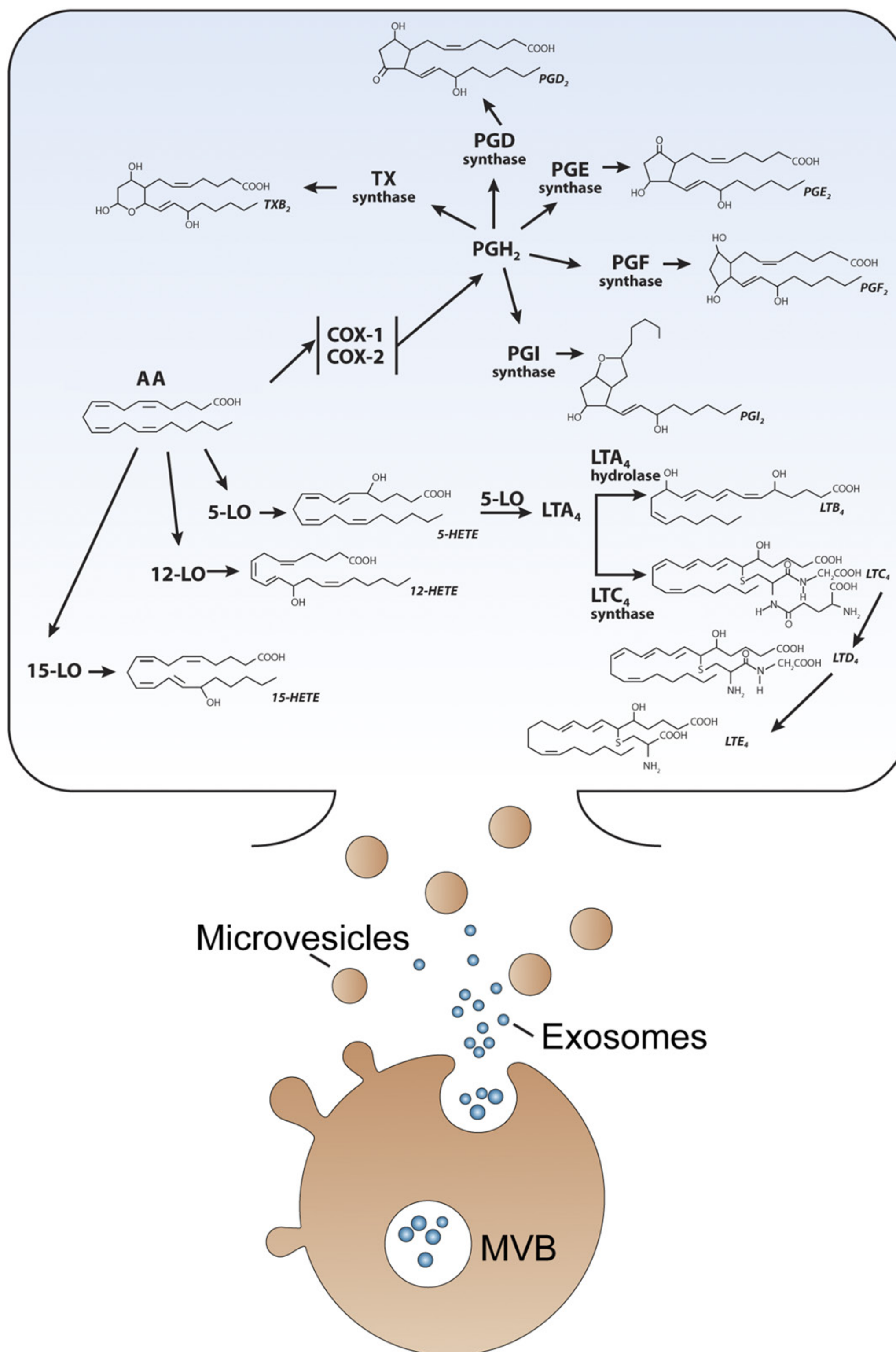


Fig. 1. Eicosanoids and the associated enzymes are comprised in EVs. Cells produce different types of EVs, such as exosomes and microvesicles. Exosomes are stored in multivesicular bodies (MVBs) and released on cell activation, whereas microvesicles are generated by plasma membrane budding and shedding. Graphic representation of the metabolism of AA into eicosanoids. AA, arachidonic acid; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; TX, thromboxane; 12-LO, 12-lipoxygenase; 5-LO, 5-lipoxygenase; LT, leukotriene; PG, prostaglandin; HETE ; hydroxyeicosatetraenoic acid.

of coagulation factors (14). Similarly to EVs circulating in blood, procoagulant activity was determined for EVs from various sources, including tumors, semen, cerebrospinal fluid, synovial fluid, and those present in saliva (18–24). However, the discovery of small noncoding RNA, the microRNA (miRNA), and the subsequent demonstration of

actual transfer of miRNA from donor cell to recipient have greatly stimulated research on EVs as mediators of intercellular communication under physiological and pathological conditions (25, 26). As current research on EV-mediated intercellular communication is skewed toward understanding the miRNA contribution, the role of other members of the EV cargo is frequently overlooked.

Notwithstanding the potential of EV-associated miRNAs as biomarkers and their importance in the regulation of mRNA stability, the EV cargo is vast and includes other components (i.e., in addition to miRNA) that can be utilized as biomarkers and can explain the roles of EVs (27, 28). Hence, studies have identified cytokines, enzymes, growth factors, functional organelles (e.g., proteasome, mitochondria) and transcription factors in EVs, which are likely to play their part in EV-mediated functions (27–31).

With their phospholipid content, EVs represent a source of esterified fatty acids that can be released by phospholipases (32–34). The signaling molecules derived from arachidonic acid (AA) and other polyunsaturated fatty acids are called eicosanoids (32, 33). It is well established that eicosanoids are implicated in multiple biological functions, such as asthma, cancer, hemostasis, immunity, inflammation and reproduction (32, 33). Their biosynthesis implicates enzymatic and nonenzymatic processes (key processes illustrated in Fig. 1), which are conserved within EVs and can be induced by enzymes present in the EV bathing milieu (35). Hence, EVs are a highly potent source of eicosanoids such as prostaglandins (PGs) and leukotrienes (LTs) (35) with demonstrated activity in vitro and in vivo. The goal of this review is to highlight the importance of the eicosanoids pathway as shuttled by EVs derived from viable cells (exosomes and microvesicles).

PLATELET-DERIVED EVs

Platelet-derived EVs, originally called platelet dust and better known as platelet microparticles, account for the majority of circulating EVs in blood (17, 27, 36). Platelets release both exosomes and microvesicles, and can also shed EVs from the tip of long membrane protrusions when they are adhered on the endothelium and in the presence of blood flow (37–39). Given that megakaryocytes, the bone marrow cells that produce platelets, also release EVs, it has been suggested that the majority of the EVs in blood under healthy conditions actually originate from megakaryocytes (39–42). Platelet-derived EVs were the first to be reported. Thus, it is not surprising that the first demonstration of EV-mediated intercellular communication also involved EVs from platelets.

One key limiting factor in eicosanoid biosynthesis is the AA availability. Phospholipase A_2s (PLA_2s) are enzymes that hydrolyze phospholipids in the *sn-2* position, thus liberating lysophospholipid and fatty acid (34, 43). If the released fatty acid is AA, it can be metabolized into eicosanoids. While some PLA_2s are intracellular, others are secreted by cells and as such are ideally positioned to interact with EVs in the extracellular milieu. The family of secreted PLA_2s ($sPLA_2$s) includes 10 small (~14 kDa) soluble proteins (44, 45) classified into different groups according to their sequence homology, structure, and number and position of disulphide bonds (45). Secreted PLA_2s from groups IIA, V, and X are among the most abundant and active enzymes (46). They present distinct substrate specificities, supporting the notion that they might not be isozymes (46–49).

Most, if not all, biological fluids, including blood/plasma, bronchoalveolar lavage fluid, cerebrospinal fluid, saliva, semen, synovial fluid, tears, and urine, contain both $sPLA_2$s and EVs, suggesting a potential interaction between EVs and $sPLA_2$s in vivo (23, 50–60). Hence, studies were undertaken to determine whether EVs could be used as substrates by $sPLA_2$. Pioneer work by Barry and colleagues (61, 62) revealed that $sPLA_2$ could release AA from platelet-derived EVs. AA transported by platelet-derived EVs was also capable of inducing mitogen-activating kinase cascade in a monocyte cell line EVs, which led to PG production (61). EVs might also contain intracellular PLA_2s, such as cytosolic PLA_2 (35), but these enzymes might not be as efficient at releasing AA from EVs than $sPLA_2$; active caspases are present in EVs (63, 64), and given that caspases can cleave intracellular PLA_2 (65), it might mitigate their activity in EVs.

Interestingly, it was found that cyclooxygenase and thromboxane synthase, contained in EVs, could metabolize AA into thromboxane, thereby promoting platelet activation and aggregation (62) (**Fig. 2**). Treatment of platelets with $sPLA_2$ did not induce thromboxane production or aggregation, demonstrating a unique function for platelet-derived EVs that is absent in platelets (62). Thus, this seminal study demonstrates that enzymes are present in EVs and are capable of metabolizing lipid substrates into potent eicosanoids. An additional mechanism leading to platelet aggregation was also identified when platelet (and erythrocyte)-derived EVs were treated with a combination of $sPLA_2$ and sphingomyelinase (66). In this case, EVs released lysophosphatidic acid, a novel lipid mediator at that time that has recognized high potency on platelets and endothelial cell functions (66).

The AA liberated from $sPLA_2$-treated EVs is also utilized by endothelial cells in a paracrine manner (62). In this case, prostacyclin is promptly produced by endothelial cells (62), suggesting that this pathway may counterbalance the activation of the vasculature triggered by thromboxane (Fig. 2). Note that although platelets can release AA in addition to unstable PGH_2, which can also be metabolized in a paracrine manner by nearby cells independently of EVs (67), these in vitro experiments were the first to identify the role of potent lipid mediators shuttled by EVs in intercellular communication.

Evidence of concerted activities of $sPLA_2$ and platelet EVs in intercellular communication was verified in vivo in the context of autoimmune arthritis (31). Platelet EVs were first identified in blood, but their small dimensions likely explain their presence in the extravascular milieu, such as in the synovial fluid of rheumatoid arthritis patients (30, 51, 52, 68–70) and in lymph (71). In rheumatoid

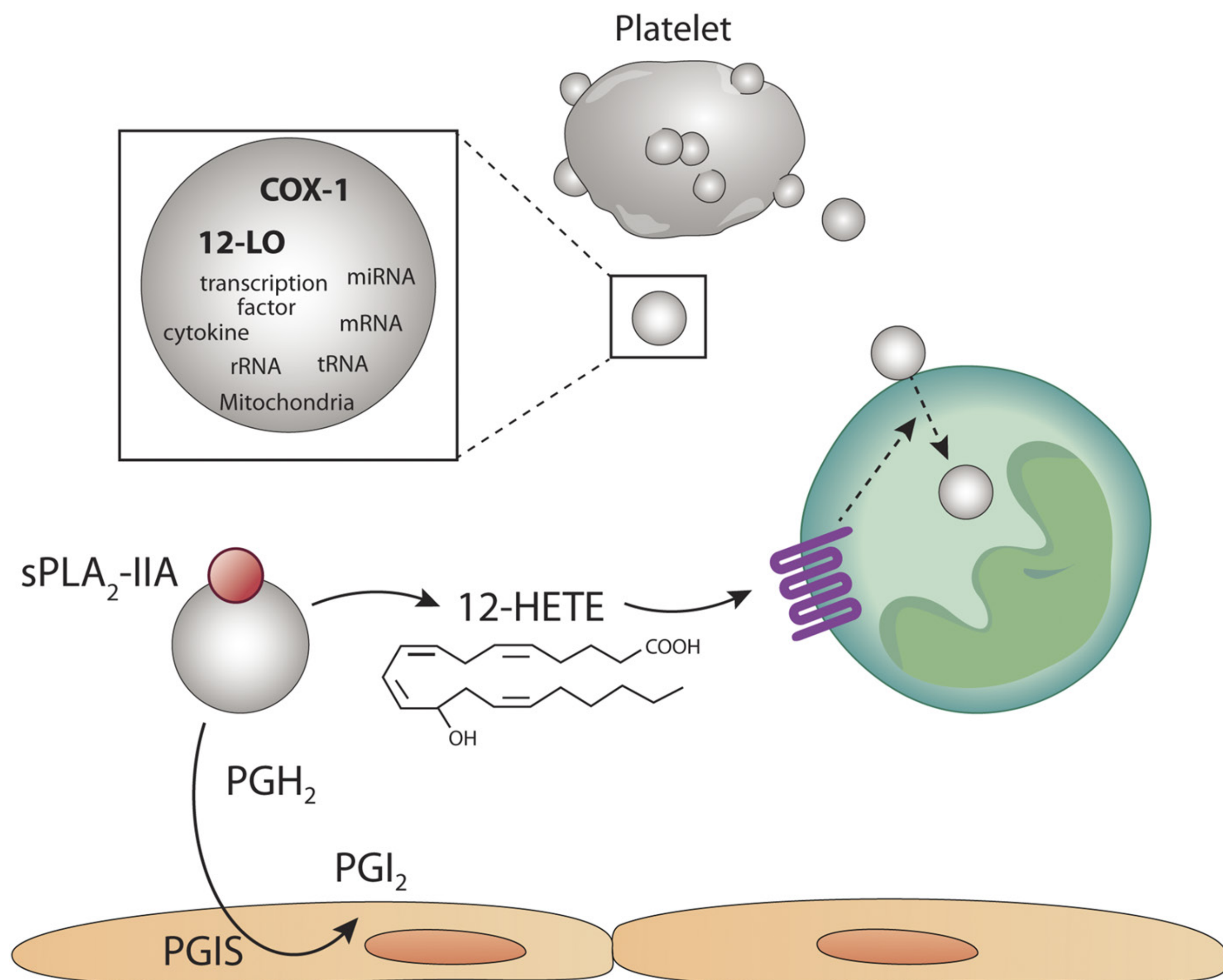


Fig. 2. Platelet-derived EVs participate in intercellular communication through their eicosanoid content. Platelets are highly proficient at releasing EVs, such as microvesicles (microparticles). EVs from activated platelets contain a broad cargo, which includes cytokines, transcription factors, cytokines, mitochondria, and nucleic acid. Of importance is the content in active cyclooxygenase-1 (COX-1) and 12-lipoxygenase (12-LO). In the presence of secreted phospholipase A_2 (sPLA$_2$) in an inflammatory milieu, platelet-derived EVs generate arachidonic acid, which can be metabolized into prostaglandin H_2 (PGH$_2$) and subsequently into prostacyclin (PGI$_2$) by the PGI synthase (PGIS) in endothelial cells. AA liberated by platelet-derived EVs can also undero lipoxygenation by the 12-LO itself present in platelet-derived EVs. Moreover, exogenous AA can be metabolized by platelet-derived EVs by 12-LO. 12-hydroxyeicosatetranoic acid (12-HETE) is generated and promotes the internalization of platelet EVs by neutrophils, thereby permitting efficient transfer of cargo. The internalization of platelet-EVs is therefore tightly regulated by the eicosanoid 12-HETE.

arthritis, neutrophils predominate among other leukocytes in the inflamed joint fluid and display a prolonged lifespan and reduced migratory activities (72). These observations point to the accumulation of factor(s) in rheumatoid arthritis that promote neutrophil plasticity (72). Thus, it was hypothesized that factors conveyed by platelet EVs could reprogram neutrophils in rheumatoid arthritis.

In rheumatoid arthritis, sPLA$_2$-IIA is overexpressed in joint lubricating synovial fluid and amplifies the disease (51, 73). Given the fact that sPLA$_2$-IIA uses EVs as a substrate (44, 62, 66), it was verified whether platelet EVs were internalized in neutrophils and whether sPLA$_2$-IIA could impact the internalization process. In this study (31), the authors utilized platelet-derived EVs, which, according to the centrifugation used and EV characterization, corresponded to microvesicles. Hence, in addition to EV markers such as those from the ESCRT system, the EVs contained miRNA, transcription factors, and sometimes even mitochondria (31). Interestingly, platelet EVs promptly associated with neutrophils, but remained on the neutrophil surface unless sPLA$_2$-IIA was present. With the involvement of sPLA$_2$-IIA, approximately 20 and 40 EVs localized in the neutrophil cytoplasm near the endoplasmic reticulum, Golgi apparatus, and lysosome within 30 and 60 min, respectively (31), pointing to the high efficiency of the process (Fig. 2).

In this study, Duchez et al. (31) conducted a complete lipidomic analysis of platelet EVs and examined the lipid mediators produced when in the presence of sPLA$_2$-IIA. It was found that the product of 12-lipoxygenase (12-LO),

12(S)-HETE, is the dominant eicosanoid produced. Hence, platelet EVs shuttle 12-LO, and the incubation of EVs with exogenous AA preferentially leads to 12-HETE production, not thromboxane (31). Therefore, these observations illustrate that while platelets can predominantly produce the eicosanoids thromboxane and 12(S)HETE, in contrast, platelet EVs mainly generate 12(S)HETE. This unique feature of platelet EVs may be explained by the suicide inactivation of thromboxane synthase in EVs (74). Using 12-LO-deficient platelets and a pharmacological approach, the study then confirmed that 12(S)-HETE is the mediator generated by platelet EVs that dictates internalization in neutrophils and permits cargo transfer (31). While the intravenous injection of fluorescently labeled 12-LO$^{+/+}$ platelet EVs in arthritic mice localized inside neutrophils in the disease joints, 12-LO$^{-/-}$ platelet EVs failed to accumulate inside neutrophils (31). Thus, intercellular communication from platelets to neutrophils is under the control of eicosanoids produced by EVs and implicates 12(S)-HETE. As the process requires sPLA$_2$-IIA, this suggests that this pathway is highly regulated and occurs when neutrophils reach an inflammatory fluid, rich in sPLA$_2$-IIA (Fig. 2). Whether this pathway is conserved among other cells is unknown, but given that 12-LO expression is mainly restricted to platelets (and platelet EVs), and given the involvement of the 12(S)-HETE receptor (BLT$_2$) in the internalization process (31), it suggests that this mechanism is meant to promote internalization of platelet EVs, specifically in BLT$_2$-expressing cells, such as neutrophils (75). As BLT$_2$ is reported to contribute to arthritis (76), it is also tempting to speculate that its role is mediated by EV-derived 12(S)-HETE. Moreover, it is reported that 5(S)-12(S)-HETE is the most abundant eicosanoid in the synovial fluid of rheumatoid arthritis patients (77). However, no cells are known to be capable of producing this molecule because none express both 5-lipoxygenase (5-LO) and 12-LO. Given that platelets are devoid of 5-LO, but package 12-LO in their EVs (31), the transfer of 12-LO-containing EVs into 5-LO-expressing neutrophils may take place in synovial fluid and account for the presence of this unique eicosanoid.

The activity of sPLA$_2$ toward the EV membrane is not unique to EVs derived from platelets. The sPLA$_2$-IIA, V, and X enzymes were shown to be capable of hydrolyzing EVs, with different potency, from erythrocytes, thymocytes, endothelial cells, and those present in semen (prostasomes) (78). The hydrolysis by sPLA$_2$ can generate fatty acids, but does not lead to clearance of EVs, as they remain detectable even in fluids rich (> 10 μg/ml) in sPLA$_2$, such as in the synovial fluid of rheumatoid arthritis patients (51, 78). When mitochondria, comprised in certain EVs and released concomitantly with EVs, are hydrolyzed by sPLA$_2$-IIA, there is generation of lyso-cardiolipin (30, 79). While cardiolipin is a phospholipid observed in bacterial membrane and mitochondria and is a recognized damage molecular pattern (80), the role of lyso-cardiolipin is unknown, but the products of the hydrolysis of mitochondrial membrane by sPLA$_2$-IIA induces the production of neutrophil extracellular traps by neutrophils (30).

MYELOID CELL-DERIVED EVs AND LUNG INFLAMMATION

Proteomic and lipidomic approaches were undertaken to determine the machinery involved in eicosanoid synthesis in EVs from the RBL-2H3 cell line, which shares characteristics with both mast cells and basophils (13). Subra et al. (13) examined well-characterized exosomes isolated by differential centrifugation, and identified phospholipase C, phospholipase D, and three classes of PLA$_2$, namely, the cytosolic calcium dependent cPLA$_2$, calcium independent iPLA$_2$, and sPLA$_2$. Moreover, addition of GTP to exosomes induced the activation of PLA$_2$ activity, elegantly demonstrating that enzymes encapsulated in exosomes can undergo further activation. Consistent with the presence of cyclooxygenase-1 and -2, prostaglandins were also detected in exosomes (13). Given that exosomes efficiently accumulated in endosomal compartments in an autocrine manner in RBL-2H3 (13), these observations suggest that the high (micromolar) concentrations of eicosanoids shuttled by exosomes may indeed mediate biological responses.

Leukotrienes are extremely potent lipid mediators involved in the pathogenesis of asthma and lung inflammation (81, 82). In a study of EVs produced by macrophages and dendritic cells (DCs), it was found that exosomes bear the necessary pathways for LT synthesis, including the 5-lipoxygnease activating protein, 5-LO, LTA$_4$ hydrolase, and the LTC$_4$ synthase (83) Similar observations were made in the study of circulating exosomes present in plasma (83). DCs and DC-derived exosomes both generate LTC$_4$, a potent cysteinyl leukotriene involved in lung inflammation, when incubated in the presence of exogenous LTA$_4$ (83). Unexpectedly, while macrophages preferentially produced LTB$_4$, the exosomes from these macrophages promptly metabolized exogenous LTA$_4$ into LTC$_4$ (83). As lung epithelial cells can metabolize exogenous LTC$_4$ into LTD$_4$, the most potent mediator of bronchoconstriction, it can be suggested that exosomes shuttling LTC$_4$ participate in this process (84). In contrast to platelet-EVs, which efficiently generate lipoxygenase products (31), exosomes from macrophages and DCs were poor producers of lipoxygenease products from AA (83), suggesting that the presence of LTA$_4$-producing cells (or EVs) is limiting in this process. Together, these data reinforce the notion that the major eicosanoid production pathways found in exosomes can differ from those present in the producing cell.

Neutrophils are also an important source of heterogeneous EVs with reported bactericidal and inflammatory roles (85, 86). Moreover, they secrete LTB$_4$, a highly potent neutrophil chemoattractant (81, 87, 88). In order to mediate its chemoattractant function, LTB$_4$ must form a stable gradient. However, it has been established that LTB$_4$ only forms transient gradients as a result of rapid diffusion due to its small size (89, 90). Studies on gradient formation of lipid modified *Drosophilia* morphogens, or the formation of palmityolated-Wnt gradients in *Drosophilia* embryogenesis and cAMP gradients in *Dictyostelium*, suggest a role for vesicles in the formation of gradients (91–93). Thus, it was

hypothesized that neutrophils could also form an LTB_4 gradient through EVs (94).

Majumdar et al. (94) determined that the majority of 5-LO is localized in multivesicular bodies in neutrophils chemotaxing toward the chemotactic peptide N-formylmethionyl-leucyl-phenylalanine, which mimics the formylated peptides released by bacteria. Multivesicular bodies associated with 5-LO are located near the nucleus (94), which is consistent with observations made in RBL-2H3 cells (13). The authors performed an extensive characterization of EVs using centrifugations on gradients, electron microscopy and biochemical approaches, and confirmed that exosomes were the EVs transporting 5-LO, in addition to 5-lipoxygnease activating protein, LTA_4 hydrolase, and LTB_4 (94). EVs, rich in LTB_4, efficiently formed a gradient chemotactic response, and knocking down Rab27a or a neutral sphingomyelinase 2 using shRNA in a neutrophil-like cell line reduced exosome secretion, the release of LTB_4, and directional motility (94). This breakthrough finding demonstrates that in order to mediate its chemoattractant activity, LTB_4 must be released within EVs (**Fig. 3**). Furthermore, as neutral sphingomyelinase 2 depletion led to a near complete inhibition of exosome release, these intriguing observations suggest that exosome biogenesis in neutrophils mainly involves a ceramide-dependent mechanism and takes place independently of the ESCRT system.

Although LTB_4-containing EVs may act directly on neutrophils in both an autocrine and paracrine manner, neutrophil-derived EVs were also demonstrated to impact other neighboring lineages (95). The interaction of platelets and neutrophils is critical to the efficient innate immune response to bacterial infections (96, 97). The interactions of platelets and neutrophils implicate P-selectin and glycoprotein Ib (GPIb) on the platelet side, and P-selectin glycoprotein ligand-1 and Mac-1 on the neutrophil side (98–100). Fibrinogen can also bridge platelets and neutrophils together through glycoprotein IIbIIIa and Mac-1 (101, 102). Rossaint et al. (95) observed that platelet GPIb stimulates the release of EVs from neutrophils through Mac-1. Neutrophil-derived EVs were shown to be rich in AA content, and were internalized in platelets in a process involving GPIb and clathrin into compartments where cox-1 is localized (95). This process results in a very potent generation of thromboxane A_2 by platelet cox-1, employing the AA from neutrophils shuttled by EVs (95) (Fig. 3). Of importance is the necessity for thromboxane A_2 in neutrophil intravascular crawling and extravasation, required to combat lung infection. Hence, the depletion of platelets or the blockade of their interaction with neutrophils using a GPIb-blocking antibody reduced survival of mice infected with *Escherichia coli* that could be rescued by intravenous injection of neutrophil-derived EVs (95). Neutrophils release EVs containing LTB_4 and its enzymatic machinery, but the blockade of GPIb did not impact the production of LTB_4, suggesting that platelets are not involved in LTB_4 synthesis in lung inflammation (95). Although these studies confirm that neutrophil-derived EVs are potent shuttles of eicosanoids that enable efficient innate immune response, it is not established whether platelet-derived EVs also shuttle eicosanoids to neutrophils in the context of lung inflammation.

TUMOR-DERIVED EVs

Tumor cells modify their environment in order to escape immune system surveillance and to promote growth (103). During tumor growth, there is expansion of myeloid-derived suppressor cells (MDSCs), thereby promoting tumor progression (104, 105). MDSCs accumulate in secondary lymphoid organs, blood, and the tumor itself and provide stroma and immune evasion (104, 105). How the tumor stimulates the generation of MDSC in the marrow is unclear, and studies were undertaken to determine whether intercellular communication through EVs was implicated (106). Exosomes, enriched by differential centrifugation from a tumor removed 21 days postinjection of a murine mammary adenocarcinoma cell line into mice, were injected intravenously into recipient mice (106). While they stimulated the generation of MDSC, assessed using $Gr\text{-}1^+$ and $CD11b^+$ markers, exosomes did not impact T-cells, natural killer cells, or B-cells (106). Exosomes even increased the size of tumors, pointing to their role in tumor growth through stimulation of MDSC. Of importance is that the authors identified PGE_2 as a key molecule shuttled

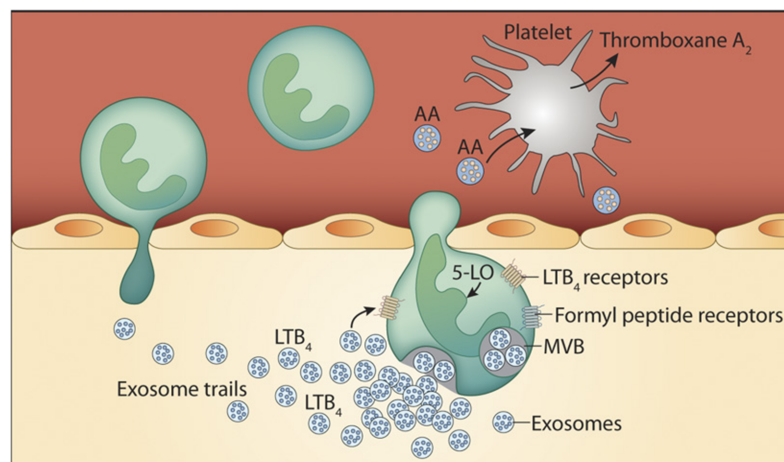


Fig. 3. Neutrophils produce extracellular vesicles rich in eicosanoid components. When neutrophils are activated by the bacterial peptide N-formylmethionyl-leucyl-phenylalanine, they release exosomes containing leukotriene B_4 (LTB_4), which form a gradient. The gradient of LTB_4 forms an "exosome trail" that extends from the EV-producing cell and is necessary in neutrophil recruitment to the site of inflammation. EVs from neutrophils also contain arachidonic acid (AA), which upon capture by platelets, is metabolized into thromboxane A_2 (TxA_2). Thromboxane A_2 generated through the intercellular communication between neutrophils and platelets promotes inflammation and pathogen clearance in lungs in a bacterial infection model. 5-LO, 5-lipoxygenase; MVB, multivesicular body.

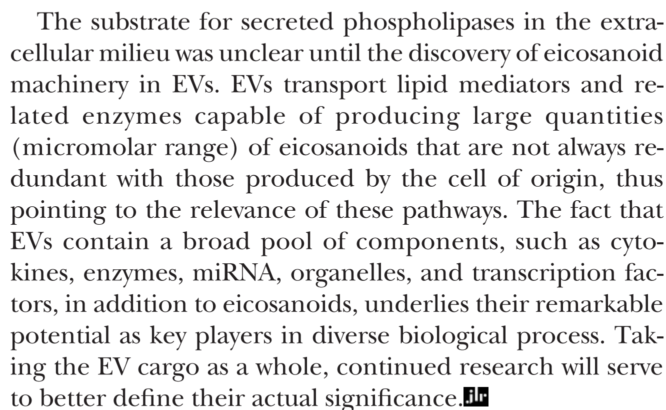
by exosomes involved in MDSC accumulation, and demonstrated that a PGE_2-neutralizing antibody could impair expansion of MDSC (106).

Tumor-derived EVs can also directly impact cells from the immune system (103). EVs liberated by the intestinal epithelium in the mucus can migrate to the liver where they induce natural killer T-cell anergy (107). Anergy was induced by PGE_2 transported by EVs and efficiently protected against liver inflammation in a hepatitis model when administered by gavage with EVs from the intestinal epithelium (107). Moreover, the microbiome in the intestine, more particularly the enterobacterium *Bacterioides fragilis,* mediates release of EVs from the epithelium, which convey sphingosine-1-phosphate and PGE_2 (108). Sphingosine-1-phosphate and PGE_2-containing EVs then recruit Th17 cells in the intestine (108). Of relevance to cancer is that Th17 recruitment, induced by EVs, favored the establishment of tumors in spontaneous and transplanted colon cancer mouse models (108). While these observations demonstrate a role for eicosanoids transported by EVs in the downregulation of the immune system in cancer, it is unknown whether similar mechanisms occur in the resolution phase of inflammation.

CONCLUSIONS AND PERSPECTIVES

Whereas lipids form the basis of the EV structure and affect EV stability or clearance in the whole organism (1, 27, 109, 110), EVs also shuttle bioactive lipid mediators and enzymes involved in their synthesis (27, 35). As the eicosanoid pathway differs from one cellular lineage to another, the fact that cells can exchange eicosanoid machinery can lead to generation of completely novel eicosanoid molecules. Furthermore, the examination of anti-inflammatory eicosanoids, such as the resolvins and lipoxins, in EVs may permit the identification of subtypes of EVs implicated in the resolution of inflammation (111). The appreciation of eicosanoid-containing EVs will lead to the discovery of novel molecules and functions for these lipid mediators and may shed light on eicosanoid pathways present in the extracellular milieu unique to certain pathological conditions.

Colossal efforts have been undertaken in recent years in order to better characterize EV subtypes (i.e., microvesicles vs. exosomes) and to ensure their authenticity (1, 10, 112). Certain markers have been proposed, but no perfect methodologies exist to easily discriminate EV subtypes and identify their compartment of origin. Determining how eicosanoids and enzymes are secreted may reveal new secretion pathways, independent of the canonical ESCRT system described thus far. How eicosanoids and associated enzymes are packaged in EVs is not well understood, but an understanding of the process may shed light on lipidomic approaches to further characterize EVs. For instance, mass spectrometry or infrared spectrometry (113) could be applied to differentiate subclasses of EVs. These approaches can also be utilized to determine eicosanoid-based biomarkers associated with EVs in certain pathologies.


The substrate for secreted phospholipases in the extracellular milieu was unclear until the discovery of eicosanoid machinery in EVs. EVs transport lipid mediators and related enzymes capable of producing large quantities (micromolar range) of eicosanoids that are not always redundant with those produced by the cell of origin, thus pointing to the relevance of these pathways. The fact that EVs contain a broad pool of components, such as cytokines, enzymes, miRNA, organelles, and transcription factors, in addition to eicosanoids, underlies their remarkable potential as key players in diverse biological process. Taking the EV cargo as a whole, continued research will serve to better define their actual significance.

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