Platelets and platelet extracellular vesicles in hemostasis and sepsis

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ABSTRACT

Platelets, cell fragments traditionally thought of as important only for hemostasis, substantially and dynamically contribute to the immune system's response to infection. In addition, there is increasing evidence that externally active platelet entities, including platelet granules and platelet extracellular vesicles (PEVs), play a role not only in hemostasis, but also in inflammatory actions previously ascribed to platelets themselves. Given the functions of platelets and PEVs during inflammation and infection, their role in sepsis is being investigated. Sepsis is a condition marked by the dysregulation of the body's normal activation of the immune system in response to a pathogen. The mechanisms for controlling infection locally become detrimental to the host if they are applied systemically. Similar to cells traditionally ascribed to the immune system, including neutrophils, lymphocytes, and macrophages, platelets are instrumental in helping a host clear an infection, but are also implicated in the uncontrolled amplification of the immune response that leads to sepsis. Clearly, the function of platelets is more complicated than its simple structure and primary role in hemostasis initially suggest. This review provides an overview of platelet and platelet extracellular vesicle structure and function, highlighting the complex role platelets and PEVs play in the body in the context of infection and sepsis.

PLATELETS IN HEMOSTASIS

Platelets are small anuclear cell fragments that circulate in the blood. Circulating platelets are discoid-shaped and smooth, with an uncompromising cytoskeleton that maintains its integrity even under the high fluid shear forces produced by blood flow.^{1–3} The traditional role of platelets is to detect and rapidly respond to blood vessel injury.^{1,4,5} On injury, vascular endothelial cells and connective tissue cells release soluble factors that attract and activate platelets.^{1,5} Platelets quickly arrive at and attach to the site of injury. Attachment leads to changes in platelet shape; they flatten, elongate, and extend small projections.^{1,2,6,7} These adaptive changes in structure enhance the activated platelets' ability to attach, spread, and recruit additional platelets to the site so that a platelet plug forms and covers the damaged area.^{1,5}

Signal transduction during platelet activation

Platelet structural changes rely on the signal transduction that occurs when platelets are activated. Platelets activate after exposure to soluble platelet agonists.^{1,5,7,8} Agonists are released from damaged cells, already activated platelets, or other inflammatory cells.^{1,2,5–11} Common agonists include collagen, ADP, von Willebrand factor (vWF), thrombin, fibrinogen, fibronectin, serotonin, and platelet-activating factor.^{1,5,7,8} When an agonist binds to a receptor on the platelet surface, alterations in intracellular signaling promote cytoskeletal rearrangements and increased platelet receptor affinity that is necessary for platelet aggregation and the formation of thrombi.^{5,12–15} Intracellular signaling occurs in three stages: (1) early platelet activation signaling, (2) intermediate common signaling events, and (3) integrin signaling.^{1,5,7,8}

The interaction of an agonist with a platelet adhesion receptor initiates early platelet activation signaling.^{1,5,7,8} Depending on the bound receptor, early platelet activation signaling occurs through the Src family kinases, phosphoinositide 3-kinases, immunoreceptor tyrosine-based activation motif,^{1,5,6} or through heterotrimeric G proteins.^{1,5} Regardless of the type of receptor involved in early activation signaling, all signal transduction pathways converge during the second stage of signaling.^{1,5} During this intermediate stage, Phospholipase C is activated,^{1,5} leading to increased calcium and activated protein kinase C.^{1,5} Both increased calcium and PKC are required for granule secretion from activated platelets and calcium is necessary for the cytoskeletal rearrangements that change the activated platelet shape.^{1,3,5}

The final stage of platelet activation signaling, integrin signaling, can be inside-out or outside-in. Inside-out signaling changes the ligand-binding function of cell adhesion proteins known as integrins.^{1,13,15–17} In the resting platelet, integrins are present on the cell surface in the low-affinity state but switch to the high-affinity state when the platelet is activated.^{5,8,12,13,15,17–19} This alteration in the affinity of integrins enhances the aggregatory function of activated platelets, allowing them to bind to other platelets and cells at the site of injury.^{5,12,13,15,17} Outside-in signaling is initiated solely when the agonist-bound receptor is the integrin GPIIb/IIIa.^{5,12,13,15,17,20,21} When an agonist binds to GPIIb/IIIa, changes in the

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Figure 1 Platelet function in infection. When platelets interact with pathogens, such as bacteria, they activate and degranulate. Contents released during degranulation promote death of the pathogen and activation of the innate and adaptive immune systems. Antimicrobial molecules, including reactive oxygen species, microbicidal proteins, and kinocidins, can directly kill pathogens. Platelets also release, among other factors, CD40 and CD154 to attract B cells, complement factors C8 and C9 to ultimately form part of the membrane attack complex (MAC), and platelet factor 4 (PF4) to attract monocytes, T cells, and neutrophils to carry out their traditional roles as players in the immune system. Neutrophils that are directly activated by platelets and/or endotoxin form neutrophil extracellular traps (NETs), web-like structures created when neutrophils release their granular and nuclear contents into the extracellular space. NETs help trap pathogens to minimize their spread from the primary site of infection. Spread of pathogens is also minimized when platelets bind to damaged endothelium and, when combined with fibrin, form microthrombi to create a physical barrier.

intracellular domains of the integrin allow a G protein subunit to bind.^{5 12 13 15–17 20–22} This amplifies the platelet's responses to G-coupled-protein receptor (GPCR) agonists, enhancing activation signaling^{5 12 13 15–17 20 22} and ultimately promoting cytoskeletal rearrangement and activated platelet function.^{5 12 13 16 17 20}

PLATELETS AS AN IMMUNE 'CELL' IN INFECTION AND SEPSIS

In addition to their well-established role in hemostasis,^{14–6} activated platelets participate in the host immune system in a variety of ways, including direct interaction with an invading pathogen, mediation of the inflammatory response via the complement and innate immune systems, and communication with leukocytes and the adaptive immune system (figure 1).^{6 10 23–29} Platelets also play an important role in the immune response when the normal activation of the immune system is dysregulated, as in sepsis.³⁰

Platelet interactions with invading pathogens

Because platelets are abundant in the blood and highly sensitive to changes in their environment, they frequently interact with invading pathogens directly and before the activation of the innate immune response.^{1 23 25–28} Platelets

have been shown to directly phagocytose bacteria, fungi, viruses, and protozoa.^{6 10 23 25–28} Microorganisms are internalized into vacuoles when platelets are activated via the FcγRIIa receptor.^{1 23 25–28} Similar to primary immune cells, platelets also express toll-like receptors (TLRs) that allow them to respond to pathogen-associated molecular patterns (PAMPs).²⁵ In addition to TLRs, platelets use the GPIIb/IIIa receptor to form fibrin bridges to adhere to microbes and the Fc receptor FcγRIIa to bind to IgG immune complexes to form platelet–pathogen aggregates.²⁵

Platelets activated by invading pathogens also undergo degranulation of antimicrobial molecules, including reactive oxygen species (ROS), platelet microbicidal proteins, and chemokines with microbicidal activity, referred to as kinocidins.^{1 25–29} Platelet microbicidal proteins are small, cationic polypeptides that can kill pathogens, including fungi, via direct disruption of the cell membrane through voltage-dependent channels.^{1 25 31} Platelet microbicidal proteins are stored in platelet granules and in the cytoplasm of platelets.²⁵

The effectiveness of antimicrobial molecules expressed by platelets is enhanced by the formation of microthrombi. Microthrombi form through the interaction of activated platelets and the vascular endothelium. As innate immune cells are activated, tissue factor (TF) is released into the circulation from monocytes and macrophages³² and the coagulation pathway is initiated. This results in the formation of fibrin, which forms a clot when it interacts with platelets at the primary site of infection.^{32–37} The resulting matrix of fibrin and platelets provides a physical barrier to the spread of infection and creates a concentrated area of platelet antimicrobial molecules.

Neutrophil extracellular traps (NETs) are another mechanism by which the immune system tries to maintain a pathogen at the primary site of infection. NETs form when neutrophils bind to endotoxin-exposed platelets or endotoxin alone.^{32–34 38} Under these conditions, neutrophils release their granular and nuclear components into the extracellular space.^{32–34 38} A web-like structure is formed by DNA and proteins, trapping bacteria.^{32–34 38} In this way, NETs serve to limit the spread of pathogens from the initial site of infection and kill the invading pathogen.^{32–34 38}

Platelet mediation of the innate immune response

Platelets further contribute to the host's defense by enhancing and activating components of the immune system, including the complement system.^{1 23 25–28} As part of the innate immune system, the complement system is primarily responsible for the direct lysis and opsonization of pathogens for phagocytosis, recruitment of immune cells to the site of infection, and activation of the adaptive immune system.²⁴ The complement receptor C1q is expressed on activated platelets and facilitates the activation of the classic complement pathway by binding the complement protein C1q.²⁴ Activated platelets increase expression of C3a and C5a receptors, which bind their respective anaphylatoxins, increasing vascular permeability and allowing for the translocation of immune cells from the vasculature to the active site of infection.²⁴

Platelets also contain terminal complement factors C8 and C9, which are released on activation of the platelet.^{24 25} After their release, C8 and C9 combine with the activated C5b, C6, and C7 to form the C5b-C9 complex, also known as

the membrane attack complex (MAC).^{1 24 35 36} The complex causes cell lysis through the formation of transmembrane channels.^{1 24 35 36} MAC also induces vascular endothelium to release TF and vWF, which are strong initiators of the coagulation cascade. The activation of coagulation leads to microthrombi formation, as previously discussed, and the eventual activation and degranulation of platelets.^{1 24 35 36} Thus, a positive feedback loop exists between the complement system and platelets, where platelets perpetuate the complement system and the complement system activates additional platelets. This enhances the elimination of pathogens, but also predisposes the host to thrombosis.

Activated platelets also release chemoattractants, such as platelet factor 4 (PF4), which attract innate immune cells like neutrophils and monocytes to the site of infection.^{1 23 25–27} Further, platelets potentiate the effect of neutrophils, important first responders in innate immunity. The direct interaction of platelets and neutrophils is primarily mediated through the binding of P-selectin on the surface of activated platelets with P-selectin glycoprotein ligand-1 (PSGL-1).^{1 25 35} These platelet-bound neutrophils release antimicrobial proteins and lysozymes and show increased respiratory burst, leading to enhanced phagocytosis, bacterial clearance, and degranulation.^{1 35 37}

Platelet mediated enhancement of the adaptive immune system

Platelets interact with the adaptive immune system by recruiting and activating T cells and B cells.^{1 25–27 35} Platelets attract T cells to the site of infection through PF4.^{1 25–27 36} They also express co-stimulatory proteins, which induce differentiation, cytokine production, and activation of T cells. Additionally, platelets process antigens and present them to T cells via major histocompatibility complex class I molecules.^{1 25–27 35} Platelets directly activate B cells via the release of soluble CD40 and CD154 ligands, leading to proliferation, differentiation, isotype switching, and memory B cell generation.^{1 25–27 35 39} In a study by Sprague *et al*, when platelets from wild-type mice were transfused to CD154 deficient mice during a viral infection, there was a significant increase in circulating virus-specific IgG levels, providing evidence of a link between platelets and the adaptive immune system.⁴⁰

Platelets in sepsis

Platelets contribute to the pathology of sepsis. Activated platelets and increased coagulation lead to disseminated intravascular coagulation (DIC) and clots in the microvasculature, which can lead to cell death and organ dysfunction.^{41–50} Indeed, platelets accumulate in the microvasculature of many of the commonly failing apoptotic end organs in sepsis, such as the lungs, liver, and spleen, limiting adequate blood flow and drainage to and from these organs.^{31 51–53} In sepsis, platelets that have been activated by thrombin or lipopolysaccharide (LPS) express increased amounts of transcript-1 (TLT-1) receptor.⁴⁸ TLT-1 has been shown to facilitate platelet aggregation, therefore augmenting clot formation and promoting DIC.

Platelet–neutrophil interactions also contribute to complications in sepsis. As discussed, endotoxin-activated platelets activate neutrophils to release NETs. This enhances

bacterial clearance, which benefits the host; however, host endothelial cells are also damaged in this process and NET formation can lead to liver damage in vivo.^{32 34 38 54} Additionally, neutrophil-platelet aggregates release thromboxane A2 (TXA2), an arachidonic acid metabolite that activates endothelial cells via G protein-coupled thromboxane receptors. Receptor activation leads to integrin activation, platelet aggregation, and increased vascular permeability.³⁷ The activation of the pulmonary vascular endothelium by TXA2 is known to be integral in the development of acute lung injury (ALI) associated with sepsis.³⁷ Blocking platelet-neutrophil aggregation by blocking P-selectin not only decreases circulating levels of TXA2, but in a sepsis-induced murine model of ALI, improves gas exchange and leads to prolonged survival.³⁷

Although platelets are anuclear, they do contain a large messenger RNA (mRNA) reservoir and can regulate the translation of their transcriptome based on external activating conditions, including sepsis.^{1 55–57} Specifically, genes related to cell adhesion, chemotaxis, and inflammatory and immune response are differentially expressed in septic platelets.⁵⁸ The cell death-inducing enzyme granzyme B, a serine protease, is an example of a selectively transcribed protein.⁵⁸ Granzyme B induces programmed cell death by proteolytic cleavage of key substrates in target cells^{59–63} and its mRNA is upregulated in platelets of septic patients, suggesting that platelets could be cytotoxic in sepsis.^{58 66 67} The mechanism by which septic platelets release granzyme B remains unclear; there are multiple externally active platelet entities that could transport the protein to the target cell.

EXTERNALLY ACTIVE PLATELET ENTITIES—PLATELET GRANULES AND EXTRACELLULAR VESICLES

In addition to direct interactions, platelets communicate with other cells indirectly through the release of externally active entities, such as granules and extracellular vesicles. Platelet granules are intracellular vesicles that fuse with the plasma membrane to release their contents on activation, while platelet extracellular vesicles (PEVs) are membrane-bound entities that are released into the extracellular space on platelet activation (figure 2).^{68–70}

Platelet granules

Granule exocytosis from activated platelets helps with the control of endothelial damage, as highlighted by the fact that deficiencies in granule secretion result in bleeding defects.^{5 71–74} The three main types of platelet granules are: alpha granules, dense granules, and lysosomes.^{1 71 73} Platelets have approximately 40 to 80 alpha granules per platelet, ranging from 200nm to 500nm in diameter.^{1 71 75 76} Alpha granules contain large proteins and molecules, like growth factors and chemokines.^{1 71 75–77} Dense granules are ten times less abundant than alpha granules and are smaller. They are identified by the presence of electron-dense spherical bodies within the granule.^{1 71 73 78} Dense granules contain smaller molecules such as ADP, ATP, calcium, magnesium, and serotonin.^{1 71 78} Lysosomal granules are the least common type of granule within platelets, with only a few lysosomes per platelet.^{1 71} Lysosomal granules contain lysosome-associated membrane proteins, acid hydrolases, and cathepsins.^{1 71} While

Figure 2 Release of externally active platelet entities—platelet granules and extracellular vesicles. Platelet granules are intracellular vesicles that fuse with the plasma membrane to release their contents. Alpha granules, the most abundant type of platelet granule, contain large proteins and molecules and can also contain endosomally-derived PEVs. Endosomally-derived PEVs are the smaller of the two types of PEV and contain variable, non-random proteins, mRNA, and miRNA. Plasma membrane-derived PEVs, the second type of PEV, are usually larger than those that are endosomally-derived and they bud off of the plasma membrane. They generally contain protein cargo, including functional platelet receptors, multiple kinds of RNA, and even cell organelles. PEVs often share the same antigens as the parent platelet. mRNA, messenger RNA; miRNA, microRNA; PEVs, platelet extracellular vesicles.

their role is not fully understood, they may play a role in clot remodeling and further platelet activation.¹

Platelet Extracellular Vesicles

As previously mentioned, PEVs are membrane-bound entities that are released into the intracellular space on activation of the parent platelet. PEVs are abundant in circulation, representing the majority of extracellular vesicles in the blood.⁷⁹ Extracellular vesicles can be further divided into two major groups: plasma membrane-derived microvesicles and extracellular vesicles of endosomal origin, commonly referred to as exosomes.^{70–82} Previously, extracellular vesicles were also differentiated based on size, with the larger, plasma membrane-derived ‘microparticles’ classified as ranging from 100nm to 1000nm in diameter, compared with the smaller endosomally-derived ‘exosomes’ at 40nm to 100nm in diameter.^{70–80–81–83–84} Given an inconsistent use of terms in the literature and difficulty in demonstrating specific biogenesis pathways, the International Society for Extracellular Vesicles (ISEV) has recently updated their position on nomenclature.⁸² Instead of using terms such as ‘microvesicles’, ‘exosomes’, or ‘microparticles’, the ISEV endorses the use of the term ‘extracellular vesicles’ (EV), which can further be described based on size, biochemical composition, and cell of origin.⁸²

Platelets release EVs when stimulated by physiological agonists and mediators, such as chemokines, apoptosis, or increased shear stress.^{68–70} The type of PEV and its

contents differ based on the stimuli to which the platelet is exposed.^{83–85} EVs contain membrane and cytosolic proteins, including fully functional receptors, circular RNA (circRNA), long non-coding RNA (lncRNA), microRNA (miRNA), mRNA, and even encapsulated cellular organelles.^{85–86–88–90}

Formation of PEVs

On activation of the platelet, plasma membrane-derived PEVs bud off from the plasma membrane, thus sharing the same antigens as their parent platelet, such as CD41 and CD62p.^{70–83} Given their larger size, plasma membrane-derived PEVs are more likely to contain protein cargo and they express and transfer functional receptors from platelet membranes to other cell types.^{80–83–91}

PEVs of endosomal origin are generally smaller than the plasma membrane-derived PEVs.^{80–83} Endosomally-derived PEVs are released when multi-vesicular bodies fuse with the platelet plasma membrane.^{70–84–92} They are often stored in alpha granules and are released when the granules fuse with the plasma membrane.^{70–83} Similar to plasma membrane-derived PEVs, the cargo of endosome-origin PEVs is variable, non-random, and can include proteins, mRNA, and miRNA. Although the contents of endosomally-derived EVs can be specific to the parent cell, their cargo does not necessarily reflect the contents of the cell from which they were released. For example, endosomally-derived EVs from certain cancer cells contain increased amounts of proteins, mRNA, and miRNA specific to increasing angiogenesis, cell cycle disruption, and immune suppression, thus supporting tumor spread and proliferation.⁹³ Given this finding in cancer cell EVs, it is possible that PEVs contain platelet-specific cargo and/or cargo not typically found in platelets.

PEVS IN HEMOSTASIS

PEVs are integral to the regulation of the coagulation system with both procoagulation and anticoagulation properties. The coagulation capacity of PEVs is estimated between 50 to 100 times that of platelets alone. They carry the prothrombotic proteins annexin-V, factor X, and prothrombin.^{80–89–94–96} The increased coagulation capacity of PEVs is due, in part, to an increase in surface area exposing phosphatidylserine, a negatively charged molecule typically found on the intracellular surface of the platelet plasma membrane, which is exposed on the surface of PEVs.^{89–95–96} In the resting state, the enzyme flippase acts to maintain aminophospholipids, specifically phosphatidylserine, on the inner leaflet of the platelet membrane.⁹⁵ In the activated state, flippase activity decreases and floppase activity increases, leading to an imbalance of phosphatidylserine on the outer surface of the platelet membrane prior to PEV formation.⁹⁵ By facilitating the interaction between factor Va and factor X, the phosphatidylserine on PEVs provides the basis for the prothrombinase complex, leading to an increase in thrombin production.^{89–95–97}

Healthy individuals’ PEVs also express highly concentrated CD41 on their surface, which generates small amounts of thrombin via TF and factor VII independent pathways.⁹⁸ These quantities of thrombin in turn activate protein C, a natural anticoagulant, implying that PEVs may not only be procoagulants, but are also involved in the regulation of coagulation.⁹⁸

PEVS IN INFECTION AND SEPSIS

Similar to platelets, PEVs contribute to the activation of host immune cells by attracting leukocytes to areas of pathogen invasion, facilitating leukocyte-to-leukocyte interactions, and inducing the release of specific cytokines.^{81 99–101} In fact, some of the proinflammatory actions traditionally ascribed to platelets may actually be due to PEVs. For example, the inflammatory reaction seen in some cases of platelet transfusion is likely mediated by PEVs.^{81 101} PEVs contribute to inflammation in infection through direct recruitment of leukocytes, including T cells, B cells, and monocytes, via chemokine release and facilitate the interaction between monocytes and endothelial cells by binding P-selectin and PSGL1.¹⁰² PEVs can upregulate the expression of cyclooxygenase-2, which is the first step in converting arachidonic acid into prostacyclin, causing vasodilation and allowing for an increase in inflammatory cells reaching the site of infection.¹⁰³

As previously mentioned, PEVs' surface markers and their contents vary based on the platelet-activating stimulus. LPS and staphylococcal superantigen-like protein (SSL) are both PAMPs that directly stimulate PEV formation when co-incubated with platelets from healthy subjects, via activation of platelet TLRs.^{86 104} Although a specific fungal stimulant has not been identified, sepsis secondary to fungal infections appears to trigger a PEV release with differential surface markers compared with sepsis triggered by bacteria.¹⁰⁵

LPS stimulates platelets via the TLR4 pathway, leading to c-Jun N-terminal kinase and Akt activation, as it would in nucleated cells, resulting in increased IL-1 β in secreted PEVs.¹⁰⁴ The released IL-1 β induces the expression of vascular cell adhesion protein 1 on endothelial cells, which promotes the binding of leukocytes to the endothelium.¹⁰⁴

PEVs released in response to SSL5, a *Staphylococcus aureus* specific antigen, bind to monocytes and lead to a dose-dependent increase in the release of proinflammatory cytokines IL-1 β and tumor necrosis factor- α .⁸⁶ SSL5 binds to the platelet membrane receptor glycoproteins GPIIb, GPIIb/IIIa, or GPVI to stimulate the release of PEVs. These PEVs in turn bind to and stimulate CD14+ CD16+ monocytes via the P-selectin–PSGL1 and CD40L–CD40 interactions and activation of the NF κ B inflammatory pathway.⁸⁶

In addition to being proinflammatory, PEVs released in response to *Neisseria meningitidis* also express increased amounts of TF on their surface, contributing to the sepsis-associated increase in coagulation.¹⁰⁶ The activation of platelets in *N. meningitidis* infection occurs indirectly through the activation of the complement system by the bacteria. Specifically, C5a is associated with an increase in both platelet-associated and PEV-associated TF. Blocking C5 decreases PEV-associated TF expression and reduces TF-dependent procoagulant activity in coagulation assays.¹⁰⁶

In viral infections, the PEV count is found to be elevated and contributes to the activation of the host's immune system.^{91 107 108} In immunized hosts exposed to H1N1, platelets are stimulated to release PEVs both through the formation of thrombin and by the binding of Fc γ RIIA.¹⁰⁷

PEVs also contribute to the propagation of the virus itself. In patients with HIV, PEVs appear to facilitate entry of HIV into target cells through C-X-C chemokine receptor type 4 (CXCR4).⁹¹ HIV requires the cell surface antigen

CD4 and a chemokine receptor, either CXCR4 or C-C chemokine receptor type 5, to enter its target cell. While all of these receptors are present on cells of hematopoietic lineage, specifically lymphoblasts, HIV has also been shown to infect CXCR4 negative cells such as endothelial cells, astrocytes, and cardiomyocytes. Rozmyslowicz *et al* demonstrated that PEVs can transfer CXCR4, a common receptor on platelets, to CXCR4 negative cells, making them vulnerable to infection with HIV.⁹¹

In Dengue virus infections, PEVs have not only been shown to be predictive of clinical outcomes, but appear to heavily contribute to the pathogenicity of virus.^{108–110} Platelets are activated to release PEVs directly by Dengue virus via the tyrosine-kinase receptor CLEC-2. PEVs, in turn, activate neutrophils and macrophages through the CLEC5A receptor, inducing NET formation and the release proinflammatory cytokines.¹⁰⁸ Sung *et al* found that blocking the effect of PEVs on leukocytes in a mouse model of Dengue increased survival from 30% to 90%, implicating PEVs in the severe virulence of the disease.¹⁰⁸

The release of PEVs and their contents in sepsis is not only influenced by the activating stimulus, but can also be affected by host platelet receptors. Children with a GPVIa receptor haplotype had a two-fold increase in circulating PEVs compared with those with GPVIb receptor haplotype in a study of children with sepsis.¹¹¹ The number of PEVs was found to be higher in children with GPVIa receptor and these individuals were also more likely to present with severe features of septic shock, including significant organ failure, compared with those with the GPVIb haplotype.¹¹¹ In addition to contributing to our knowledge of an individual's personal response to an infection, these findings further highlight the role of PEVs as mediator of the inflammatory response to infection.

PEVs as predictive markers

There has been some investigation into the use of PEVs as a predictor of illness and illness severity in sepsis. The number of PEVs appear to be overall lower in sepsis non-survivors and in those with DIC; however this effect is mitigated when PEVs are adjusted for overall platelet count.¹¹² Conversely, Boscolo *et al* found that the PEV to platelet ratio was higher in septic patients that required longer support with vasoactives, mechanical ventilation, and those that developed DIC.¹¹³ This difference is noted at the time of diagnosis and persists during the course of the illness.¹¹³

Direct organ dysfunction secondary to small PEVs in sepsis

Though the smaller PEVs remain understudied compared with larger PEVs, there is evidence that they contribute to the proliferation of inflammation and organ dysfunction in sepsis.^{114–116} The evidence to date suggests small PEVs are involved in myocardial dysfunction and endothelial cell apoptosis, prominent features of sepsis.

In sepsis, vascular endothelial and smooth muscle cell apoptosis is mediated by the generation of ROS through the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathway.¹¹⁷ Janiszewski *et al* demonstrated a predominance of small PEVs, compared with small EVs of other inflammatory cell origin (monocytes, granulocytes,

and lymphocytes) in a study of adults with early sepsis.¹¹⁴ Further, PEVs obtained from septic patients not only contain two cytochrome subunits of the NADPH oxidase, but are also present at twice the concentration in PEVs of septic patients compared with healthy controls.¹¹⁴ Incubation of endothelial cells and vascular smooth muscles cells with these PEVs induces enhanced spontaneous superoxide generation and significantly increases the rate of apoptosis of those cells in vitro. This suggests an important role for small PEVs in the vascular dysfunction seen in sepsis via a redox signaling pathway.^{114 115}

PEVs also depress the function of cardiac myocytes in sepsis, possibly contributing to low cardiac output, a hallmark of septic shock.¹¹⁶ Rabbit hearts exposed to PEVs of septic patients show signs of myocardial dysfunction based on a decrease in myocardial contractility. Myocardial cells exposed to PEVs of septic patients demonstrated a higher level of nitrate and nitric oxide, providing a possible pathway of the observed myocardial dysfunction.¹¹⁶

CONCLUSION

Considering their simple structure as anuclear cell fragments, platelets and their extracellular entities play a complex and important role in regulating basic homeostatic and inflammatory functions. Directly and indirectly, through PEVs, platelets contribute to the activation and regulation of coagulation and inflammation in the body. Beyond the 'sticky cell fragments' necessary for clot formation, our understanding of platelets has evolved to include them as immune 'cells'. Further, the role of the platelet in the regulation of both hemostasis and inflammation in the healthy state and dysregulation in the disease state contribute to our overall knowledge of the pathogenesis of disease.

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