Microparticles: markers and mediators of sepsis-induced microvascular dysfunction, immunosuppression, and AKI

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Abstract

Sepsis is a severe and complex syndrome that lacks effective prevention or therapeutics. The effects of sepsis on the microvasculature have become an attractive area for possible new targets and therapeutics. Microparticles (MPs) are cell membrane-derived particles that can promote coagulation, inflammation, and angiogenesis; and can participate in cell-to-cell communication. MPs retain cell membrane and cytoplasmic constituents of their parental cells, including two pro-coagulants: phosphatidylserine and tissue factor. We highlight the role of microparticles released by endothelial and circulating cells after sepsis-induced microvascular injury, and discuss possible mechanisms by which microparticles can contribute to endothelial dysfunction, immunosuppression, and multi-organ dysfunction--including sepsis-AKI. Once viewed as cellular byproducts, microparticles are emerging as a new class of markers and mediators in the pathogenesis of sepsis.

Keywords

Introduction

Sepsis is characterized by a complex systemic response to an overwhelming infection that may lead to multi-organ dysfunction, including acute kidney injury (AKI). Sepsis is the dominant cause of AKI in the ICU, accounting for nearly 50% of episodes [1, 2]. Despite several decades of extensive study, there are no therapies or preventative agents beyond fluids and antibiotics. Even supportive care studies, including goal directed volume therapy and albumin infusion have not shown clinical benefit [3, 4]. Many potential pathways and multiple drug targets have been identified in animal models of sepsis; however, the translation from animal to human studies has been quite challenging [5-7]. While older studies were focused on inflammation and global renal blood flow, more attention has been given recently to renal microvascular alterations (i.e., capillary leak, leukocytes and platelet

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adhesion with endothelial dysfunction, microthrombi formation) and immunosuppression that occur during sepsis and sepsis-AKI.

The microvascular alterations and endothelial dysfunction that occur during sepsis resemble the endothelial dysfunction that happens in many chronic conditions with the healthy endothelium shifting to a damaged pro-coagulative and pro-inflammatory phenotype [8]. Recent epidemiologic and mechanistic studies suggest that AKI and chronic kidney disease (CKD) are not distinct entities but are rather closely interconnected: CKD is a risk factor for AKI, AKI is a risk factor for the development of CKD, and both are risk factors for cardiovascular disease [9]. The link between an acute episode of AKI and a possible future chronic loss of kidney function and/or cardiovascular disease may be explained, in part, by the microvascular injury that often occur in both acute and chronic conditions. Several mediators and processes take part in the microvascular dysfunction that occurs during sepsis-AKI. We briefly review some aspects of this syndrome and highlight the role of microparticles, cell membrane-derived particles that may play a critical role in both the initiation and propagation of sepsis [10].

Sepsis, microvascular dysfunction, and oxidative stress

In the vascular microcapillary bed, circulating cells interact with highly dynamic endothelial cells through a variety of receptors and elaborated mediators whose functions include vasoregulation, coagulation, barrier maintenance, immune cell recruitment and oxygen transport [11]. Microvascular dysfunction, defined as any damage to the microvascular cellular components, including endothelial cells, smooth muscle cells and circulating blood cells, is often detected by altered flow or adhesive properties [11]. During sepsis, microvascular dysfunction can occur by several mechanisms: 1) blood flow stagnation from altered circulatory cell function (loss of reticulocyte flexibility, increased leukocyte adhesion, etc); 2) endothelial cell injury; 3) parenchymal cell injury with oxygen utilization abnormalities and mitochondrial dysfunction; and 4) increased coagulopathy (clotting factors, protein C, tissue factor, etc) [12-14]. Also, severe capillary leakage can result in interstitial edema exacerbating low tissue oxygen perfusion, contributing to hypoxia and multi-organ dysfunction [12, 15, 16].

In vivo studies in animal models using intra-vital video microscopy visually demonstrate impaired arteriole and capillary microcirculation in several organs during sepsis [17-20], including the kidneys [21-23]. During sepsis, a decrease in functional microcapillary density, as defined as the length of continuously perfused microvessels per observation area, is associated with increased heterogeneity of microvascular perfusion, due to the presence of intermittently or non-perfused capillaries nearby well-perfused capillaries. This is a dynamic process, as non- or poorly-perfused capillaries may become perfused a few minutes later. These alterations have been shown in several preclinical models of sepsis in several vascular beds [24, 25]. Besides microcirculatory flow changes, endothelial cells also change their phenotype with an increased expression of adhesion molecules (becoming more pro-inflammatory), and tissue factor (becoming more pro-coagulant) [26].

Because of tissue hypoxia caused by microvascular dysfunction during sepsis, parenchymal cells can switch from aerobic to anaerobic respiration, producing toxic byproducts such as

reactive oxygen species (ROS). In an anaerobic state ROS are aggressively produced by the mitochondria, resulting in more cell damage and endothelial cell dysfunction, perpetuating a vicious cycle [27-29]. Oxidative stress and microvascular dysfunction together have an important role in the development of sepsis-AKI. The relationships between renal microvascular changes and ROS generation have been studied in preclinical models of sepsis, using live animal intra-vital video microscopy. These elegant studies have demonstrated that increased tubular generation of ROS and peroxynitrite occur following a decline in peritubular capillary perfusion (secondary to microvascular dysfunction) [21, 30, 31]. Microvascular dysfunction during sepsis causes important micro-environment changes that have deleterious effects not only locally, but also systemically, and its contribution to multi-organ dysfunction including AKI is significant. Therefore, understanding the microvascular derangements during sepsis is essential for future development of biomarkers and therapeutics in this complex disease.

The role of microparticles in microvascular dysfunction, multi-organ dysfunction, and sepsis-AKI

Sepsis-induced microvascular injury causes the release of microparticles (MPs) into the systemic circulation. MPs are cell membrane-derived particles, 0.2 to 2μm in diameter that promote coagulation and inflammation [32] (Figure 1), perpetuating microvascular injury. For example, when human neutrophils were activated with a calcium ionophore to induce MPs release, these MPs induced loss of cell membrane integrity and caused other morphological changes in human umbilical vein endothelial cells [33]. MPs contain proteins and lipids from cell membranes and cytoplasm of their parental cells, and are generated from a wide variety of cells, including endothelial cells, red blood cells, monocytes, and platelets. The outer leaflet of the MPs membrane contains two pro-coagulants: phosphatidylserine, a pro-coagulant phospholipid, and tissue factor. As their internal cargo includes proteins, mRNAs, and miRNAs, MPs have recently been shown to participate in a novel form of cell-cell communication [34]. The actions of MPs may depend on their cellular origin and state of activation of the parental cells. Given their multi-faceted roles in thrombosis, inflammation, and angiogenesis (Figure 2), MPs have been considered as possible culprits during the pathogenesis of sepsis and septic shock, and possibly sepsis-AKI [35]. During sepsis in mice, most of circulating MPs are derived from platelets (85%), with a minority originated from endothelial cells and monocytes. A small number of MPs during sepsis contain erythrocyte markers [36]. Studies that address the role of each particular MP sub-population on endothelial function and immune system, and their interactions, are still lacking.

Microparticles differ from exosomes mainly by their size and genesis. Exosomes are smaller (0.04 -0.1 μm in diameter) than microparticles. Whereas microparticles bud from cell membranes, exosomes are an end-product of the endocytic recycling pathway. After inside-out endocytic vesicles form at the plasma membrane and fuse to form early endosomes, these endosomes mature and become late endosomes that constitute the multivesicular bodies. These inside-out multivesicular bodies (MVBs) invaginate to produce right-side out vesicles within the MVBs, then the MVBs can directly fuse with the plasma membrane and thereby release right-side out exosomes into the extracellular space. As MPs are produced

directly through the outward budding and fission of membrane particles from the plasma membrane, their surface markers are largely dependent on the composition of the plasma membrane at the time of release; whereas, exosomes are rich in lipid raft constituents due to organelle maturation [37]. Microvesicles is a term that includes both exosomes and microparticles that are smaller than the detection limit of flow cytometry. The term microvesicles is sometimes ambiguous in the literature, reflecting the technical difficulty in purifying and/or validating the purity of microparticles, microvesicles, and exosomes.

MPs are recognized by specific receptors. Annexin I has been identified as one essential component to recognize MPs in cultured endothelial cells [38]. Annexin I binds to phosphatidylserine on the surface of the MPs. MPs produced from wild-type but not from annexin I- null polymorphonuclear cells (PMNs) inhibited IL-1beta-induced leukocyte trafficking on human umbilical vein endothelial cell (HUVEC) monolayers [39]. CD36, a class B scavenger receptor that binds multiple ligands, can also act as a receptor for endothelial cell-derived MPs during vascular injury [40]. Blocking CD36 (either genetically or with an inhibitor) improves sepsis survival and acute outcomes, including AKI, related to decreased inflammation and better granulocyte activity with better local (peritoneal) bacterial containment [41]. However, the number of other MPs receptors is unknown; some may work in combination with Annexin I, or independent of Annexin I.

MPs can be detected in the circulation in a normal/ healthy state (Figure 3a) [26, 35, 42], but are greatly increased after sepsis (Figure 3b) [36]. Because MPs circulate systemically, they can behave as pathogenic autocrine (distance) disseminators and have been implicated in the multi-organ dysfunction that characterizes sepsis and septic shock [26]. MPs can directly modulate endothelial cell nitric oxide and prostacyclin production, stimulate cytokine release and tissue factor induction, and promote monocyte chemotaxis and adherence to the endothelium [26, 42]. Systemic injection of MPs from septic rats into healthy rats reproduces the hemodynamic, inflammatory, and oxidative stress patterns of sepsis, including nitrosative stress [35]. Similarly, MPs extracted from whole blood of septic patients exerted pleiotropic and tissue-selective changes in expression of pro-inflammatory proteins related with nitrative and oxidative stresses; changes not seen when MPs were isolated from non-septic controls [43]. MPs derived from septic subjects also increased renal markers of inflammation and oxidative stress in healthy rats [43].

MPs can also contribute to the prothrombotic state in sepsis by initiating disseminated intravascular coagulopathy (DIC), a known contributor to multiple organ dysfunction (Figure 2) [36, 44, 45]. Tissue factor present on the surface of MPs is a primary initiator of coagulation. The activity of tissue factor associated with peripheral blood MPs is related to disease severity and bacteremia in patients with community- acquired febrile *E. coli* urinary tract infections; and MPs-tissue factor activity declines upon resolution of infection [46]. MPs contain other pro-coagulant molecules as well. Delabranche *et al*. have demonstrated in a cohort study involving 100 patients with septic shock, that elevations in endothelium-derived CD105-labeled MPs and reductions in CD31-labeled MPs are strongly associated with early DIC, and might predict DIC occurrence and early vascular injury among septic patients [47, 48]. CD105, or endoglin, is a type III auxiliary receptor for the transforming growth factor beta (TGF-β) superfamily, and is highly expressed on the vascular

endothelium in adults [49]. CD31, also known as platelet endothelial cell adhesion molecule)1 (PECAM-1), is a molecule expressed on all cells within the vascular compartment, with higher expression on endothelial cells [50].

Zafrani *et al.* demonstrated a direct role of MPs in the pathogenesis of sepsis and sepsis-AKI, on both inflammatory and coagulation pathways, using calpain signaling to modulate the number of MPs. Calpains are calcium-activated neutral cysteine proteases that play an important role in inflammatory processes and lymphocyte apoptosis [36]. Increasing calpain activity can cause platelet activation including shape change and the generation of MPs rich in phosphatidylserine [51]. Calpastatin is a specific endogenous inhibitor of activated calpain activity. In a CLP model of sepsis, transgenic mice over-expressing calpastatin had better survival and less organ dysfunction (including lung and liver damage, and sepsis-AKI), and less lymphocyte apoptosis compared with wild type mice. Calpastatin overexpressing mice also had a decreased inflammatory response and DIC, as well as a dramatic reduction in the number of circulating MPs. Furthermore, MPs transferred from septic wild type mice worsened the survival and increased coagulopathy of septic calpastatin overexpressing mice. This study demonstrates not only a deleterious net effect of calpains, but also that among all of the potential effects of calpains, MPs account for nearly all calpain-mediated injury during sepsis. The large increase in MPs during sepsis again highlight that MPs may be both a marker and mediator of sepsis [36].

Role of microparticles in immunosuppression

Only one third of patients die during the first week after sepsis. Thus, most septic patients do not die during the overwhelming inflammatory immune response phase of sepsis, but rather, they die from an increased susceptibility to secondary infections during a latter immunosuppressive state, that can last for weeks. Recent efforts have been made to better understand the pathophysiology of this late immunosuppressive phase of sepsis, with the development of possible biomarkers and therapeutic targets [52-56].

MPs may also play a role in the immunosuppression associated with sepsis. MPs shed from platelets stored for platelet transfusions can alter the function of cultured macrophages and dendritic cells toward less reactive states. Sadallah *et al.* demonstrated that human stored platelet-derived MPs reduced the release of TNF-α and IL-10 by macrophages activated by LPS or zymosan A. Further, platelet-derived MPs attenuated the differentiation of monocytes into immature dendritic cells by IL-4 and GM-CSF: immature dendritic cells lost part of their phagocytic activity and their LPS-induced maturation was down modulated when exposed to platelet-derived MPs [57]. MPs can inhibit polymorphonuclear leukocyte chemotactic responses in sepsis. Neutrophil-derived MPs can inhibit neutrophil chemotaxis *in vitro*. This inhibitory effect on neutrophils is mediated by annexin I, which binds to phosphatidylserine on the surface of the MPs. MPs produced from wild-type but not from annexin I- null PMNs inhibited IL-1beta-induced leukocyte trafficking on human umbilical vein endothelial cell (HUVEC) monolayers [39].

MPs have been found to be elevated in other conditions where both endothelial dysfunction and immune system alterations coexist, such as pre-eclampsia [58, 59]. Syncytiotrophoblast-derived MPs (STBMPs) circulate in normal third trimester pregnancy, but are present in

significantly higher concentrations during pre-eclampsia [60], and these MPs suppress T lymphocytes in vitro [61]. Circulating endothelial-derived MPs are also elevated during pre-eclampsia, and strongly correlate with proteinuria [62].

Finally, a meta-analysis of randomized trials involving 8735 patients found that a more restrictive, compared to a more liberal, strategy of red blood cell transfusion was associated with a lower risk of serious infections (pneumonia, mediastinitis, wound infection, and sepsis) [63, 64]. A possible link between stored blood cell-derived MPs with immunosuppression in patients who receive blood transfusion is an association that merits further investigation. Therefore, MPs may act as mediators and/or perpetuators of immunosuppression during sepsis.

In summary, MPs released during sepsis participate in several pathological pathways that are activated during this complex disease, and their contribution to sepsis pathophysiology is summarized in Figure 2.

Targeting microvascular dysfunction during sepsis

Because microvascular dysfunction is responsible for profound metabolic perturbations at the tissue level and contributes to sepsis-induced multi-organ dysfunction (MOD), including AKI, efforts have been made in order to find possible therapeutic targets within the microvascular system. A complex interplay between microvascular dysfunction and oxidative stress in the pathogenesis of sepsis may be more effectively targeted by drugs that interfere with both mechanisms. Several primarily anti-oxidant agents are effective in animal models of sepsis, [65] as well as drugs that target primarily endothelial dysfunction. However, drugs such as resveratrol may have a dual mechanism of action (restoration of peritubular microvasculature perfusion and reactive nitrogen species scavenging) [30]. Erythropoietin has also been shown to improve endothelial function, kidney function, and survival in experimental sepsis, through eNOS activation and anti-inflammatory effects [66-70].

Sepsis is also associated with a time-dependent increase in circulating levels of vascular endothelial growth factor (VEGF), which is a potent stimulator of endothelial permeability and involved in the proliferation, migration, and survival of endothelial cells, but also contributes to inflammation and coagulation [71, 72]. VEGF levels are elevated among septic patients, and are positively correlated with mortality [73]. Anti-VEGF antibody (Bevacizumab) has been shown to attenuate inflammation and decrease mortality in an experimental model of severe sepsis [74], and sFLT-1, an endogenous soluble VEGF receptor that neutralizes VEGF, improves survival in experimental sepsis [75, 76].

Several groups have studied the effects of progenitor or stem cells, which is one way of going beyond the classical approach of single mediators. The number of circulating endothelial progenitor cells (EPCs) is increased in the peripheral blood of septic patients, and is positively associated with better survival [77, 78], and erythropoietin is known to be a potent stimulator for endothelial progenitor cell mobilization [79]. Interestingly, septic patients with AKI (according to AKIN criteria) have a higher number of peripheral EPCs than septic patients in the "low creatinine group". Despite increased numbers of EPCs

during sepsis-AKI, these cells have a decreased proliferative capacity [78]. Stromal cell-derived factor-1α (SDF-1α) facilitates EPCs recruitment and is elevated in murine sepsis models. Recently, the effect of exogenous EPCs derived from human cord blood on murine sepsis caused by cecal-ligation puncture (CLP) was studied, as well as the role of CTCE, a SDF-1α-analogue.

Both exogenous EPCs and CTCE increased 7-day survival, and their effects were synergistic. Either EPCs alone or sub-threshold EPCs and CTCE combination administered 6h after CLP significantly increased plasma IL-10, with no effect on IL-6 or TNF-α; and also decreased lung capillary leakage as shown by the Evans blue dye assay [80]. Administration of EPCs augments plasma expression of microRNA-126 and microRNA-125-b, which can influence endothelial function [81-83]. While most of what is known regarding the role of MPs during sepsis suggests a harmful/deleterious effect, MPs may have beneficial roles; their function vary according to their cells of origin and the state of those cells. Cantaluppi *et al.* demonstrated that MPs derived from EPCs protect the kidney from AKI following ischemia-reperfusion injury while delivering a miRNA cargo (including miR-126) that can contribute to reprogramming hypoxic resident renal cells to a regenerative program, and the mice that received EPCs-derived MPs were also protected from CKD following AKI [84]. Dye from labeled EPCs-derived MPs was detected in endothelial and tubular epithelial cells 2h after intravenous injection. Further, EPCs-derived MPs have direct effects on cultured hypoxic tubular epithelial cells. These intriguing results support further exploration of the fate of circulating microparticles [85], especially in more complicated models of AKI, including sepsis-AKI.

We have demonstrated that administration of bone marrow-derived mesenchymal stem cells (BMSCs) to mice shortly (up to 1h) after the induction of sepsis increases survival and improves organ dysfunction, including AKI, by immunomodulatory effects: monocytes and/or macrophages from septic mice treated with BMSCs release more interleukin-10 (IL-10), and the beneficial effects of BMSCs were eliminated by macrophage depletion or pretreatment with antibodies specific for IL-10 [86]. Interestingly, a study by another group demonstrated that MPs derived from plasma of septic patients increases mRNA expression of IL-10 in engineered vascular tissue and increases contraction of these vascular cells induced by histamine [87]. The paracrine effects of mesenchymal stem cells (MSCs) during AKI may be driven, at least in part, by a horizontal transfer of mRNA and microRNAs through MPs. Mice subjected to unilateral ischemia-reperfusion with contralateral nephrectomy that received intravenous injection of MPs derived from human adult MSCs immediately after injury were protected from AKI and subsequent CKD onset. Pretreatment of the same MPs with RNase to inactivate associated RNA prevented their protective effects [88]. A recent paper shows that MPs from in vitro expanded kidney-derived mesenchymal stem cells contribute to recovery from AKI following ischemia-reperfusion injury by improving proliferation of peri-tubular capillary endothelial cells and decreasing peritubular microvascular rarefaction, possibly by acting as carriers of pro-angiogenic signals [89]. While these studies were performed in the ischemia-reperfusion model, it is possible that therapeutics with MPs derived from stem cells, known to have protective effects during AKI, may also have beneficial effects during sepsis-AKI.

Targeting several mediators that participate in the microvascular microenvironment and are involved in sepsis-induced microvascular injury have been shown to be beneficial in preclinical models of sepsis. The protective effects of endothelial progenitor cells or mesenchymal stem cells during sepsis and sepsis-AKI through their paracrine effects are promising. MPs may be responsible, at least in part, for these protective paracrine effects of stem-cells, supporting the hypothesis that MPs functions are strongly related to their sources (cells) of origin. Further studies are needed to better understand the individual subpopulations of MPs contributions to sepsis and sepsis-AKI.

Possible role of microparticles in amplification of sepsis by co-morbid conditions

In critically ill patients requiring mechanical ventilation, preexisting chronic kidney disease (CKD) dramatically impacts short and long term outcomes, including all-cause AKI, and 30-day and 1-year mortality [90]. We have reproduced this effect in preclinical animal models, whereby CKD amplifies the deleterious effects of sepsis, including sepsis-AKI [91, 92].

Endothelial function is severely impaired during CKD [93-97]. Circulating endothelial-derived MPs are elevated both in adults [98, 99], and in children with CKD [100], and are associated with vascular dysfunction (Figure 3c) [100]. Endothelial-derived MPs isolated from patients with CKD impair endothelium-dependent relaxation of rat aortic arteries, and decrease NO release by endothelial cells in vitro [99].

It is unknown whether MPs participate in CKD progression, or whether they contribute to the worse outcomes seen in septic patients with underlying kidney dysfunction. Conversely, severe any-cause-AKI is associated with increased risk of short-term and long-term mortality, incident CKD and accelerated progression to end-stage renal disease [101], but it is not known if circulating MPs (released from a damaged endothelium) could be involved in the development of these events, participating in kidney scarring and CKD.

Previous endothelial dysfunction associated with CKD may have an impact on therapeutic choices during sepsis. Septic mice with previous CKD (after 5/6 nephrectomy) did not respond to sFLT-1, demonstrated to be beneficial in previously healthy mice [76, 92]. The role of MPs during CKD progression, or during an 'acute on chronic' episode, are unknown at present; but may represent a future therapeutic target. A schematic view of what may happen during an 'acute-on-chronic' scenario is represented in Figure 3d.

Conclusions

New possible targets are emerging with the advances in our understanding of how sepsis affects the microvasculature. MPs, released after endothelial dysfunction during sepsis, are possible markers and contributors/ perpetuators of further endothelial dysfunction. The development of therapeutics preventing the release of MPs from injured endothelium, or directly interfering with the MPs themselves, or their receptors, may be reasonable targets in sepsis and other diseases associated with microvascular dysfunction. Besides perpetuating endothelial dysfunction and coagulation, MPs may also participate in the immunosuppression found in the late states of sepsis. Whether MPs are important as amplifiers of disease (as intercellular messengers), or biomarkers, or both, is not completely

known, and may depend on which cell types generate MPs and their state of activation. So far, harmful effects of MPs during sepsis-AKI have been demonstrated, but as a part of a natural response to infection/injury, it is likely that there are some benefits conferred by MPs during sepsis, during AKI, and possibly during sepsis-AKI. More studies are needed to better understand the effects of MPs during sepsis-AKI, especially with a deeper understanding of specific MPs sub-populations. Their functions may be intrinsically determined by their cells of origin, whereby some MPs may be harmful and others protective.

In the future, it may be possible to engineer artificial therapeutic MPs that either contain or lack specific surface molecules (thrombin, PS), or carry passenger or Trojan horse molecules (mRNA, micro-RNAs) that may be beneficial in the treatment of sepsis and sepsis-AKI. Perhaps these modified MPs could even be directly targeted to the kidneys, or a specific organ, or a specific vascular bed. This could be accomplished by manipulating specific surface ligands, or by causing upregulation of receptors in specific organs. MPs are potential therapeutic targets to prevent or treat sepsis and sepsis-AKI.

Acknowledgments

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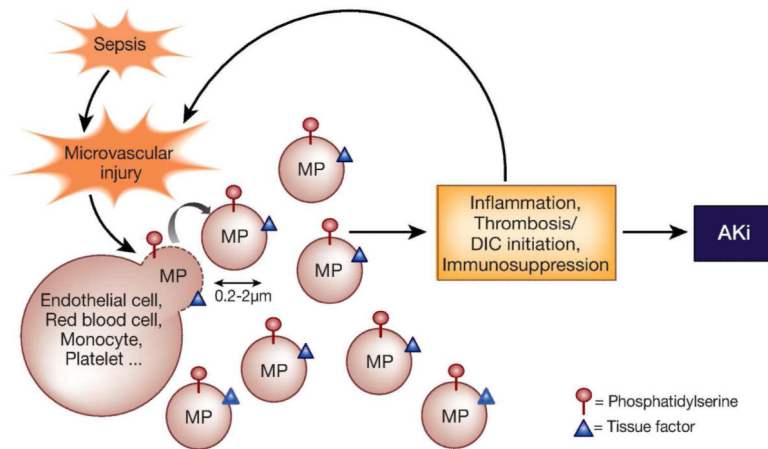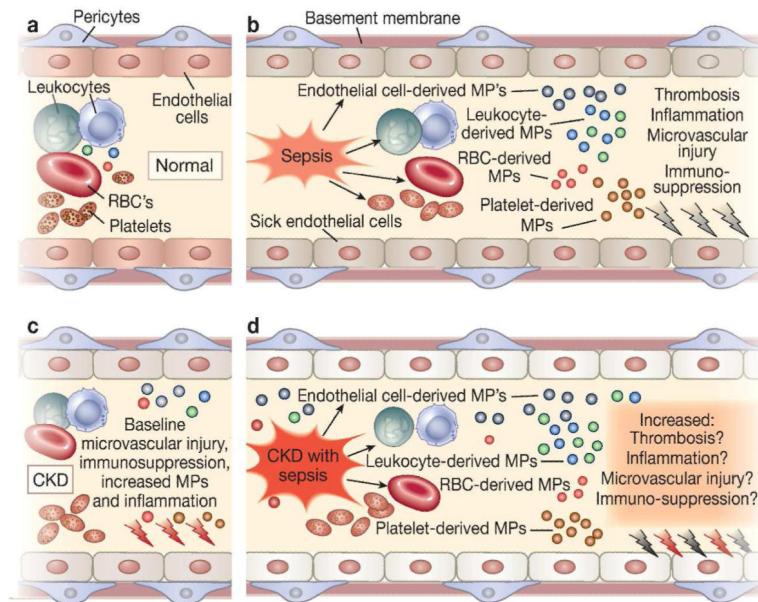
Figure 1.
Schematic view of sepsis-induced microparticles (MPs) released into the systemic circulation following microvascular injury. MPs may arise from different cell types and are between 0.2 and 2 μm in size. MPs can contribute to inflammation, DIC initiation, and immunosuppression, which contribute to multiple organ injury including acute kidney injury (AKI), and can cause further microvascular injury and MPs release.
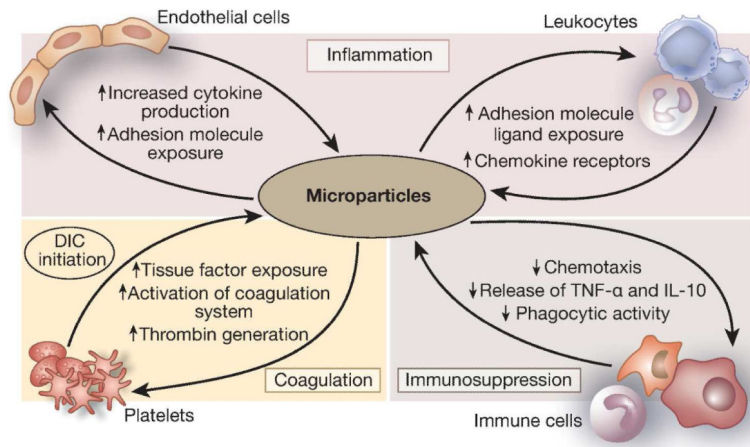
Figure 2.
Schematic representation of functions attributable to microparticles (MPs) during sepsis. During microvascular injury, endothelial cells and circulating cells such as immune cells and platelets release MPs. MPs then exert several pro-thrombotic, pro-inflammatory, and immunosuppressive actions on these same cells, perpetuating harmful cycles and microvascular injury. During sepsis, the majority of circulating MPs are derived from platelets.

Figure 3.
3a During normal/ healthy states, microparticles (MPs) are released from circulating cells in small numbers; **3b** during sepsis, endothelial and microvascular injury occur with subsequent release of MPs, which perpetuates the microvascular injury and promotes inflammation, immunosuppression, and DIC; **3c** during CKD, there is endothelial damage and increased number of circulating MPs. The role of these MPs during CKD is yet to be established; **3d** represents a hypothetical association between sepsis events (3b) in patients with pre-existing chronic disease such as CKD (3c). It is known that CKD amplifies sepsis (79). It is possible that MPs may participate in this event.