Microparticles and acute lung injury

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McVey M, Tabuchi A, Kuebler WM. Microparticles and acute lung injury. Am J Physiol Lung Cell Mol Physiol 303: L364–L381, 2012. First published June 22, 2012; doi:10.1152/ajplung.00354.2011.—The pathophysiology of acute lung injury (ALI) and its most severe form, acute respiratory distress syndrome (ARDS), is characterized by increased vascular and epithelial permeability, hypercoagulation and hypofibrinolysis, inflammation, and immune modulation. These detrimental changes are orchestrated by cross talk between a complex network of cells, mediators, and signaling pathways. A rapidly growing number of studies have reported the appearance of distinct populations of microparticles (MPs) in both the vascular and alveolar compartments in animal models of ALI/ARDS or respective patient populations, where they may serve as diagnostic and prognostic biomarkers. MPs are small cytosolic vesicles with an intact lipid bilayer that can be released by a variety of vascular, parenchymal, or blood cells and that contain membrane and cytosolic proteins, organelles, lipids, and RNA supplied from and characteristic for their respective parental cells. Owing to this endowment, MPs can effectively interact with other cell types via fusion, receptor-mediated interaction, uptake, or mediator release, thereby acting as intrinsic stimulators, modulators, or even attenuators in a variety of disease processes. This review summarizes current knowledge on the formation and potential functional role of different MPs in inflammatory diseases with a specific focus on ALI/ARDS. ALI has been associated with the formation of MPs from such diverse cellular origins as platelets, neutrophils, monocytes, lymphocytes, red blood cells, and endothelial and epithelial cells. Because of their considerable heterogeneity in terms of origin and functional properties, MPs may contribute via both harmful and beneficial effects to the characteristic pathological features of ALI/ARDS. A better understanding of the formation, function, and relevance of MPs may give rise to new promising therapeutic strategies to modulate coagulation, inflammation, endothelial function, and permeability either through removal or inhibition of “detrimental” MPs or through administration or stimulation of “favorable” MPs.

acute respiratory distress syndrome; permeability; coagulation; inflammation; endothelial function

ACUTE LUNG INJURY (ALI) as a result from either direct or indirect mechanical, toxic, infectious, or inflammatory challenges to the lung presents initially as dyspnea and hypoxemia, which can rapidly progress to respiratory failure. ALI and its most severe form, the acute respiratory distress syndrome (ARDS), are characterized by bilateral exudative chest infiltrates visible by roentgenograms with no evidence of left heart failure and a PaO2/FiO2 ratio <300 (ALI) or even <200 (ARDS) (151, 171). Within days, ALI progresses from an initial inflammatory exudative phase with a leaky edematous lung to a proliferative phase involving fibrin deposition and a concomitant decrease in respiratory compliance that finally, if the patient survives, proceeds to a fibrotic phase in which the lung is burdened with scarring as it remodels while attempting to heal. Despite the well-documented benefit of protective ventilatory strategies and increasing knowledge of the etiological mechanisms of ARDS, mortality rates remain relatively stagnant around 40% (133), with incidence estimates of up to 75 per 100,000 (171).

A broad spectrum of different cell types contribute to the pathogenesis of ARDS, including alveolar epithelial and vascular endothelial cells that undergo changes in permeability leading to edema formation and alveolarcapillary injuries, as well as platelets and immune cells such as polymorphonuclear neutrophils (PMNs), alveolar macrophages, and monocytes that are critically involved in inflammatory responses (104, 108, 170, 171). In contrast to the characteristic anticoagulant and profibrinolytic basal state of the lung, ALI is associated with a prothrombotic and antifibrinolytic shift that favors deposition of fibrin and accelerates and potentiates inflamma-
Considerable research has focused on the direct interaction and communication between different cell types both in vivo and in vitro and has revealed important pathophysiological aspects of ALI. However, a rapidly growing number of studies have recently started to recognize the critical role of microparticles (MPs) as biomarkers, communication shuttles between these different cells and cell types, and independent functional effectors in a large variety of diseases ranging from endemic pathologies such as hypertension, diabetes, and atherosclerosis to frequently lethal syndromes such as ALI (7, 16, 27, 31, 36, 44, 60, 65, 79, 99, 118, 129, 135, 138, 155, 172). Here we provide a brief overview on the main characteristics of MPs, review the current knowledge on their role in inflammatory disease with a particular focus on ALI, and oppose their proposed detrimental and beneficial effects in lung injury, thus providing a rationale for their future use as both therapeutic targets and tools.

**Microparticles**

The first accounts of MPs date back to 1967 at which time they were considered to be nothing more than cellular dust (177). In fact, MPs are tiny cell-derived, intact vesicles that are formed by partitioning off from activated or apoptotic eukaryotic cells (Fig. 1). MPs are defined by their size, which ranges between 50 nm and 1 μm, and their distinctive lipid layer composition. MPs have intact lipid bilayers, yet their outer layer has a characteristically high level of negatively charged phospholipids, particularly phosphatidylserine (PS). MPs may contain membrane and cytosolic proteins, transcription factors, genetic material such as ribosomal RNA (rRNA), messenger RNA (mRNA), and microRNAs (miRNA) as well as lipids or even organelles supplied from parent cells (22, 32, 109, 116). Occasionally, membrane vesicles of less than 0.1 μm are subcategorized as nanoparticles and have been proposed to originate from lipid rafts on parent cell membranes; accordingly, they may display distinct protein endowments and functions (reviewed in Refs. 77, 134, 155).

**Microparticles subserve intercellular information exchange.** The distinct cellular elements retained from precursor cells allow MPs to function as transcellular delivery systems for the exchange of biological signals. Notably, information exchange between MPs and target cells can occur in a bidirectional manner, thus generating a continuous and dynamic exchange of antigens, cell signaling molecules, and genetic material that opens an entire new spectrum of opportunities for tightly regulated (or dysregulated) signal propagation.

In principle, MPs can transfer information from the MP-generating donor cell to a wide range of target cells either by direct cell-cell contact for delivery of genetic material, proteins, and lipids or alternatively by remote interaction through secretion of soluble mediators and effectors by MPs. Currently, we distinguish at least four distinct mechanisms how MPs may...
interact with their target cells that are illustrated schematically in Fig. 2. First, MPs are able to stimulate outside-in signaling in target cells via presentation of membrane-associated ligands that directly bind to their respective surface receptors, or via release of secreted factors such as for example cytokines that act on the target cell via receptor-mediated or receptor-independent mechanisms (102). Second, MPs can be internalized into recipient cells, a process that has also been proposed to underlie the rapid clearance of circulating MPs from the blood (41). For example, fluorescently tagged platelet MPs (PMPs) can be endocytosed by brain microvascular endothelial cells and are subsequently detectable in endosomes and lysosomes (50). Third, MPs may fully or partially fuse with the target cell, allowing for complete or selective transfer of contents including membrane and cytosolic proteins, bioactive lipids (109, 139), or even whole cell organelles, as recently demonstrated for the microvesicular delivery of mitochondria by mesenchymal stem cells (73). Last, MPs may deliver genetic material in form of mRNA and miRNA. The encoding mRNAs can be directly translated to alter the target cell proteome (6, 45), whereas miRNAs are small noncoding RNAs that can modulate gene expression in target cells at the posttranscriptional level, in that they bind to complementary sequences on target mRNAs, commonly resulting in translational repression and gene silencing. The export of miRNA from donor cells is largely regulated by ceramide (166), a notion that gains relevance in light of the parallel implication of ceramide in MP generation (155), which may thus facilitate miRNA egress with MPs in a coordinated fashion. At the level of the target cell, subsequent miRNA entry is facilitated either by endocytosis of MPs or by fusion of their lipid bilayer with the plasma membrane (166). Lately, miRNAs have been detected in a wide variety of different MPs as generated from peripheral blood cells in humans (70) or mesenchymal (39) or embryonic stem cells (180), respectively, and miRNA transfer by MPs has been demonstrated to critically modulate target cell responses such as for example angiogenesis in endothelial cells (64).

Because miRNAs have recently been recognized to contribute to injurious mechanisms in ALI (183), transfer of miRNA from MPs to inflammatory or lung parenchymal cells may present an exciting novel pathomechanism in this disease that clearly deserves further exploration.

**Origin of microparticles.** Parent cells of MPs include but are not limited to platelets, megakaryocytes, monocytes, epithelial cells, lymphocytes, PMNs, endothelial cells, red blood cells, reticulocytes, mast cells, stem cells, astrocytes, glial cells, smooth muscle cells, and cancer cells (16, 20, 23, 139). Similar to the wide range of parent cells, MP formation can be triggered by diverse physiological, pathophysiological, or experimental stimuli as discussed in greater detail below.

Unstimulated, resting cells actively maintain their membrane asymmetry via phospholipid transporter enzymes, which localize negatively charged phospholipids on the inner leaflet, facing into the cell. When cellular homeostasis is disturbed or lost such as in apoptosis, the activities of these phospholipid flippases, floppases, and scramblases are dysregulated, leading to hastened changes in membrane composition of the inner and outer membrane leaflets. This process is a characteristic feature of MP formation, since it allows for the relocation of the PS-rich layer of the inner leaflet to the exterior leaflet (116, 155). The resulting changes in membrane composition and regular asymmetry in conjunction with processes such as intracellular calcium accumulation and calpain activation reviewed in detail elsewhere (116, 119) cause membrane blebbing and ultimately the formation of MPs (Fig. 3).

Externalization of PS is a precursor step in MP formation, allowing for a common shared surface marker for identification of many MPs that can be assayed by annexin V binding. Interestingly, however, it appears that there are MPs that do not stain appreciably for annexin V, which adds controversy to the identification and strict definition of what constitutes an MP (116, 153). Furthermore, it seems that some MPs can also be

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**Fig. 2.** Mechanisms of interactions between MPs and target cells. Interaction between MPs and their target cells can occur via essentially 4 different mechanisms: outside-in signaling via ligand-receptor interaction or secreted factors (top right); internalization and lysosomal processing of MPs facilitates for example subsequent presentation of MP antigens on the surface of target cells (bottom right); fusion-mediated transfer of surface receptors, proteins, and lipids (bottom left); and delivery of genetic material such as mRNA or miRNA (top left). MPs may fuse either completely or temporarily with target cells resulting in either complete or selective transfer of MP contents. Redrawn and modified from a figure originally published in Beyer et al. (22).
in their production. Different mechanisms of fusion between cells and MPs that could lead to phenotypic modulation of MPs and/or other cells have been recently reviewed in detail by Quesenberry and colleagues (139). A particularly striking example in this respect is the provocative hypothesis that endothelial progenitor cells may originate from the fusion of PMPs with mononuclear cells as suggested by proteomic analyses (137). Repetitive fusion events with tissue factor (TF)-expressing cells may also account for the expression of TF on various types of cells and MPs that would not otherwise express this marker. For example, platelets can become TF positive after contact with TF-expressing MPs shed from PMNs and monocytes (145). Following stimulation with endotoxin [lipopolysaccharide (LPS)] or protein kinase C activation by phorbol myristate acetate (PMA), platelets, and subsequent PMPs may obtain their TF antigens from fusion with either monocytes or monocyte microparticles (MMPs) (28). Beyond mixing of distinct lineage markers between cells and MPs there are also challenging distinctions between MPs of similar parent cell origins. Subtle variations in parent cells can be difficult to detect because of phenotypic similarities of their respective MPs, as in the case of platelet vs. megakaryocyte-derived MPs (54, 143). Hence, a series of elaborate experimental techniques have been developed to allow for the exact identification of the origin of MPs and the functional significance of these cell-derived vesicles.

**Characterization of microparticles.** In the stepwise process of experimentally isolating MPs there are considerable technical variables that can have impact on the recovery and phenotype of MPs obtained. Some of these procedural differences include the actual phlebotomy, the use of anticoagulants, storage conditions, length and number of freeze-thaw cycles, centrifugation, washing, and pelleting or filtration of MPs; and specific recommendations (Table 1) have been developed to optimize each of these steps (186). A series of review articles have scrutinized the impact of these variables that underline the sensitive and precarious nature of MP isolation and stress how caution is required to avoid undesired differences in MP isolates obtained from slight variations in handling and processing procedures (91, 134, 153, 155). The rapidly growing number of publications pertaining to MPs showcases a diverse array of techniques for preparation and isolation of MPs that is bound to generate considerable variability in terms of the final products assayed (78, 134). In recent years, this dilemma has stimulated workshops and meetings specifically dedicated to the attempt to limit variability and standardize the study of MPs; namely, the International Society on Thrombosis and Haemostasis has made strides to centralize methodological approaches for MP isolation (90).

Detection and characterization of MPs has involved strategies such as antibody-capture ELISA assays, flow cytometry, functional coagulation assays, electron microscopy (Fig. 1), atomic force or confocal microscopy, HPLC, capillary electrophoresis, and mass spectrometry (11, 24, 37, 43, 75, 77–79, 89, 91, 115, 129, 137, 153, 155, 162, 174, 181, 185). Each of these techniques comes with its specific advantages and limitations, as summarized in Table 2. Because of its general availability and versatility, flow cytometry has become the most commonly used technique for characterization of MPs and discerning their parent cell of origin depending on the expression of characteristic surface markers (Fig. 4). Most strategies with flow cy-
Flow charts of variables and current recommendations for the isolation, storage, and purification of microparticles (MPs) as proposed for platelet microparticles (PMPs) by Zwicker and colleagues (186). CTAD, citrate theophylline adenosine and dipyridamole; PFP, platelet-free plasma.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Current Recommendation</th>
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<tbody>
<tr>
<td>Venipuncture</td>
<td>needle &amp; tourniquet &gt;21-gauge needle, light or no tourniquet to avoid platelet activation</td>
</tr>
<tr>
<td>Blood collection</td>
<td>anticoagulant citrate, CTAD (as EDTA can activate platelets)</td>
</tr>
<tr>
<td>PFP preparation</td>
<td>transportation &amp; time to centrifuge avoid agitation, &lt;2 h to centrifuge (as PMPs increase over time) wide variety of speeds and times platelets may persist in PFP after a single centrifugation step</td>
</tr>
<tr>
<td>(Storage)</td>
<td>number of freeze &amp; thaw cycles −80°C, single freeze &amp; thaw cycle (multiple cycles reduce number of MPs) wide variety of speeds and times</td>
</tr>
<tr>
<td>(Further purification of MPs)</td>
<td>speed &amp; time of centrifuge further purification can reduce background signal from plasma at the risk of losing MPs</td>
</tr>
<tr>
<td>Analysis</td>
<td></td>
</tr>
</tbody>
</table>

Flow of variables and current recommendations for the isolation of MPs based on forward and side scatter characteristics as well as annexin V staining for PS in conjunction with analysis of markers characteristic for specific parent cells to test for their contribution to the individual MPs being assayed. The use of flow cytometry to this end bears a series of strengths, but also some limitations (77, 78, 89, 147, 153) that have been discussed in detail in recent literature. Proteomics presents an important alternative tool for particularly small particles not amenable to flow cytometry (117) since it can yield comprehensive information on characteristic protein expression patterns that may be utilized to identify and/or characterize MPs. This approach has been used in a series of investigations to assay different aspects of the human MP proteome (75, 96).

**Heterogeneity of microparticles.** Our current understanding of the conditions under which MPs are formed, and their individual actions, is still incomplete. Little is known about exact parameters that dictate when, how, from where, why, which, and how many MPs are formed. What is becoming evident is the vast variation in terms of genetic material and antigens MPs can express from their parent cells of origin or other cells or MPs they contact during formation or during circulation, which can be incorporated or eventually redistributed to other MPs or cells. The literature has likely not captured the full extent of variations possible since the characterization of MPs under different conditions is incomplete and we currently lack the tools to accurately assay antigens present at very low abundances on the surface of MPs. MPs come in a variety of sizes ranging from smaller nanoparticles such as those often produced by red blood cells up to MPs large enough to rival platelets (134). Similar parent cells are able to produce a spectrum of different-sized MPs depending upon whether MP formation occurs spontaneously at baseline or following stimulation (25, 74). Even cell lines such as Jurkat T cells yield a spectrum of different-sized MPs (164). Notably, this heterogeneity in size can directly impact MP surface marker expression and function as shown for platelet MPs that contain different antigens such as growth factors, chemokines, and receptors depending on their size (74). Similar to their heterogeneity in terms of origin, size, and endowment with antigens, MPs also differ widely with regard to their functional effects. MPs can shuttle information from remote cells to similar or phenotypically different cells that might never be in direct proximity. Different MPs can affect certain cells in different ways, as for example diabetics’ T cell-derived MPs can decrease endothelial nitric oxide (NO) synthase (eNOS) and increase caveolin-1 expression causing endothelial dysfunction as opposed to MPs formed by in vitro

| Table 2. Advantages and limitations of techniques commonly applied in MP analysis |
|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                | Enumeration | Sizing      | Origin      | Throughput  | Cost        | Availability |
| FCM (79,89,155,185) | +++        | +           | +++         | ++          | −           | ++          |
| ELISA (78,153,155)   | −           | −           | +           | ++          | +           | +++         |
| Functional assays (78,129,153,155) | −           | −           | +           | +++         | +           | +++         |
| EM (11,37)          | +           | +           | ++          | −           | −           | −           |
| CLM (162)           | +           | +           | ++          | −           | −           | −           |
| AFM (181)           | ++          | +           | ++          | −           | −           | −           |
| HPLC (24,43,174)    | −           | +           | +           | −           | −           | +           |
| MS (43,75,115,137)  | −           | +           | +           | −           | −           | −           |

Commonly applied techniques for the analysis of MPs and their relative strengths and weaknesses in terms of MP enumeration, MP sizing, determining the parent cell of origin, throughput, cost, and availability. AFM, atomic force microscopy; CLM, confocal laser microscopy; ELISA, enzyme-linked immune sorbent assay; EM, electron microscopy; FCM, flow cytometry; HPLC, high-performance liquid chromatography; MS, mass spectrometry. *Size fractionation.
activation of human T cells that express sonic hedgehog on their membranes and can stimulate NO production as evidenced by the improved endothelial function in a cardiac ischemia-reperfusion injury model (106, 114). Alternatively, a single MP subtype such as PMPs can interact with and recruit numerous target cells such as monocytes, NK cells, and B and T cells (114).

MP generation is triggered by a spectrum of stimuli ranging from physiological to experimental stimuli (Fig. 5). MPs can be formed constitutively by various parent cells under physiological conditions but in differing amounts, resulting in a higher abundance of circulating PMPs compared with red blood cell- or lymphocyte-derived MPs (RMP and LMP, respectively) in healthy patients (116). Additional physiological triggers for MP formation involve stresses such as heavy exercise or cyclic changes such as menstruation. MP formation in response to pathophysiological stimuli adds another level of complexity, since different triggers will stimulate MP formation from different parent cell types. Multiple simultaneous or sequential triggers can further enhance MP diversity and therefore potentially expand their range of function. For example, platelet storage conditions including platelet-rich plasma, apheresis blood bank-derived human platelets, or cold storage of platelets with and without contact with propyl gallate will produce distinct PMP variants (33, 144, 168, 178). Addition of platelet activation inhibitors such as prostaglandin E1 (PGE1), theophylline, and aprotinin during storage reduce the number of large but not small PMPs formed and modify their function in terms of platelet factor 3 activity (25). Under experimental conditions, a diverse array of potent cell stimulants such as calcium ionophores, phytohemagglutinins, LPS, N-formylmethionyl-leucyl-phenylalanine, and PMA can be utilized to in-

<table>
<thead>
<tr>
<th>MP</th>
<th>Parent Cell</th>
<th>MP Surface receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMP</td>
<td>platelet</td>
<td>CD31, CD40L, CD41a, CD42a, CD42b, CD61, CD62P, CD63, CD107a, fibrinogen, VWF</td>
</tr>
<tr>
<td>MegMP</td>
<td>megakaryocyte</td>
<td>CD41, CD184, filamin A</td>
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<tr>
<td>LMP</td>
<td>lymphocyte</td>
<td>CD3, CD4, CD8, CD11b, CD16, CD19, CD20, CD14, CD45, CD66b</td>
</tr>
<tr>
<td>MMP</td>
<td>monocyte</td>
<td>CD14, CD142, CD144, CD162</td>
</tr>
<tr>
<td>RMP</td>
<td>red blood cell</td>
<td>CD35, CD235a, IgG, complement</td>
</tr>
<tr>
<td>NMP</td>
<td>neutrophil</td>
<td>CD11a, CD11b, CD15, CD16, CD35, CD49, CD59, CD62L, CD66b, CD142</td>
</tr>
<tr>
<td>EMP</td>
<td>endothelial cell</td>
<td>CD31, CD34, CD51, CD54, CD62E, CD63, CD105, CD106, CD142, CD144, CD146</td>
</tr>
</tbody>
</table>
duce MP formation with a high efficiency and reproducibility (Fig. 5).

**Microparticles in Inflammatory Disease**

Some of the MPs characterized to date have been identified as biomarkers for a wide variety of inflammatory conditions. Elevated levels of circulating PMP, LMP, MMP, and endothelial MPs (EMPs) have been shown to accompany pathological conditions such as atherosclerosis, Type 2 diabetes, vasculitis, pulmonary hypertension, coronary disease, cardiopulmonary resuscitation, lupus, Crohn’s disease, and rheumatoid arthritis (7, 27, 28, 36, 52, 118, 129, 135, 138, 155). Likewise, systemic inflammatory states in pregnancy such as preeclampsia are associated with elevated plasma levels of MPs (146). Infec-
tious diseases are either directly associated with increased levels of MPs as seen in malaria (61) or can give rise to disproportionate inflammatory responses such as the systemic inflammatory response syndrome (SIRS), sepsis, or the multi-
organ dysfunction syndrome, that are again associated with elevated levels of MPs (129, 155). MPs have also been implicated mechanistically on several levels in the pathophysiology of sepsis, including proinflammatory signaling pathways such as target cell activation through reactive oxygen species production (ROS) and coagulopathy in terms of MP-mediated TF presentation (113).

A survey comparing 36 patients with sepsis with 18 healthier patients without sepsis showed elevated blood levels of total MPs, specifically PMPs and EMPs, whereas CD45+ LMPs were lower in patients with sepsis compared with controls (122). Interestingly, MPs from septic patients enhanced aortic contraction when transfused intravenously to mice. This effect was independent of NO modulation or cyclooxygenase enzyme expression but blocked by the specific thromboxane A2 (TXA2) antagonist SQ-29548, indicating that circulating MPs may prevent vascular hyporeactivity accounting for systemic hypotension in septic shock through a TXA2-mediated mech-

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**Fig. 5.** Triggers for MP formation from different parent cells of origin. Physiological, pathophysiological, and experimental triggers of MP formation (right) from platelets, megakaryocytes, lymphocytes, monocytes, red blood cells, polymorphonuclear neutrophils, and endothelial cells (left). APC, activated protein C; ATII, angiotensin II; autoAb, autoantibodies; C5a, complement factor C5a; C5B-9, complement factor C5B-9; CD40L, CD40 ligand; CHX, cyclohexamide; EC, endothelial cell; fMLP, N-formylmethionyl-leucyl-phenylalanine; IL-1, interleukin-1; L-NAME, L-N-gi

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**Table: Triggers of Formation**

<table>
<thead>
<tr>
<th>Parent Cell</th>
<th>Physiologic</th>
<th>Pathophysiological</th>
<th>Experimental</th>
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<tr>
<td>platelet</td>
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<td>CD40L</td>
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<td>exercise</td>
<td>collagen</td>
<td>storage</td>
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<td>menstruation</td>
<td>apoptosis</td>
<td>Ca²⁺</td>
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<td></td>
<td>pregnancy</td>
<td>C5B-9</td>
<td>TRAP</td>
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<td>basal tonic</td>
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<td>PtdIns(4,5)P₂</td>
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<td>PMA</td>
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<td>storage</td>
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<td>staurosporine</td>
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<td>monocyt</td>
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<td>red blood cell</td>
<td>ATP depletion</td>
<td>Hemolytic</td>
<td>A23197</td>
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<td></td>
<td></td>
<td>atmospheric</td>
<td>pH diamate</td>
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<td>decompression stress</td>
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<td>exercise</td>
<td>ionomycin</td>
<td>endotoxin</td>
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<td></td>
<td>L-NAME</td>
<td>C5α</td>
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<td></td>
<td></td>
<td>PMA</td>
<td>PAF</td>
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<td>APC</td>
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<td>menstruation</td>
<td>ROS</td>
<td>PAI-1</td>
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<td>pregnancy</td>
<td>IL-1</td>
<td>C5B9</td>
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<td></td>
<td></td>
<td>LPS</td>
<td>EC injury</td>
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anism (122). Furthermore, patients with SIRS have elevated levels of circulating EMPs, increased binding of MPs to PMNs, higher procoagulant activity and higher circulating levels of triggers for MP formation such as plasminogen activator inhibitor 1 (PAI-1) compared with healthy controls (126). As we discuss later in greater detail, the effects of MPs are frequently considered to aggravate the pathology in inflammatory and cardiovascular diseases, and increased formation of MPs as seen in systemic inflammatory disorders is therefore generally considered to constitute not only a biomarker, but an important pathophysiological mechanism driving the disease. However, inflammation and the associated increase in circulating MPs may also have protective effects, as pointed out by Soriano and coworkers (158), who demonstrated that increased inflammation and the presence of EMPs, PMPs, and platelet leukocyte conjugates are significantly associated with survival in septic patients. Hence, more detailed and comprehensive experimental and clinical investigations are in dire need to discern which MPs under what conditions exert beneficial or detrimental effects in inflammatory diseases.

Over the past decade, it has become evident that inflammatory diseases are closely associated with impaired coagulation and fibrinolysis, processes that can be regulated through MPs. Many but not all MPs express TF, which interacts with factor VIIa facilitating activation of factors IX and X, thus leading to generation of thrombin (103). TF MPs are characteristically found in diseases in which thrombosis is common such as cancer, sepsis, unstable angina, ALI, sickle cell disease, and thromboembolism (128, 129, 138, 165), giving rise to the speculation that they may contribute critically to the disease process. TF is a key player in the activation of the coagulation pathway, yet its action is determined not only by its expression levels, but also by its accessibility in terms of conformational state that is controversial for different cells types or conditions (128). Much of the circulating TF is in fact in an encrypted and, therefore, inactive state, whereas MPs represent a source of decrypted, active TF (127). LPS stimulates monocytes and endothelial cells to produce TF that can lead to a coagulopathy that can escalate into disseminated intravascular coagulation (103). Coincubation of platelets with monocytes and PMNs that produce high amounts of TF following stimulation with proinflammatory agents such as LPS leads to enhanced presentation of decrypted TF in the form of MPs, thus generating a prothrombotic milieu (127). Even MPs not expressing TF can still possess considerable procoagulant properties because they contain anionic phospholipids, including PS, which acts as a surface to facilitate assembly of the clotting factor machinery (103, 129, 138). Hence, MPs can be involved in coagulopathies associated with inflammatory diseases such as sepsis since they possess prothrombotic characteristics with exposed TF and PS but conversely may also activate fibrinolysis via changes in plasminogen and metalloproteases (113) or exert anticoagulant properties mediated through protein C and tissue factor pathway inhibitor (TFPI) (117). In line with the notion that MPs may have both pro- and anticoagulant effects, thrombus weight was shown to correlate with plasma levels of PMPs and older MPs in a murine thrombus model, whereas LMPs showed an inverse correlation (140).

Microparticles and acute lung injury. Several studies have shown associations of MPs with key structures and cells in ALI, giving rise to the notion that, similar to systemic inflammatory diseases, MPs may be a biomarker of the disease and play important roles by either aggravating or counteracting (or potentially both) the ongoing disease process. For example, interactions between cells such as platelets, PMNs, and endothelial cells are central to the pathology of ALI/ARDS, and MPs have been demonstrated to critically regulate these interactions and/or modify the phenotypes of the contributing cells by fusion and subsequent transfer of cellular components. In the following, we will summarize recent data in support of an association and possible functional role of MPs in ALI/ARDS and discuss potential novel interactions between MPs and lung parenchyma or circulating blood cells that are likely pertinent to lung injury.

Potential Detrimental Effects of Microparticles in ALI

Excessive inflammation, changes in coagulation and fibrin deposition, and increased permeability of the alveolocapillary barrier with resulting protein extravasation and lung edema formation are characteristic hallmarks of ALI (Fig. 6). In the following, we highlight how MPs may impact either directly or indirectly on the pathophysiology underlying these symptoms.

Inflammation. Inflammatory changes in ALI/ARDS involve an abundance of proinflammatory intra- and intercellular signaling pathways that include, but are in no way confined to, signaling via arachidonic acid (AA), TXA2, PAI-1, NO, ROS, cytokines, and protease-activated receptors (PARs). In this section, we highlight data demonstrating that MPs can modulate and/or replicate these pathways (114, 120) and thus act as proinflammatory agents promoting ALI/ARDS.

Over past years, the tight interplay between platelets and PMNs has emerged as an important aspect in the pathogenesis of ALI (100, 160, 182). Similar to platelets, circulating PMPs can interact with PMNs via linkages including glycoprotein Ib/Mac-1, β2-integrins (CD11b)/Mac-1, or P-selectin/PSGL-1 on PMPs/PMNs, thus causing PMN activation involving secretion of interleukin (IL)-8, oxidative burst, degranulation, and enhanced leukocyte rolling (79, 99). Likewise, PMPs generated from LPS-stimulated platelets promote endothelial activation as exemplified by the finding that they further increase the IL-1β-induced expression of vascular cell adhesion molecule-1 (30). Hence, circulating PMPs are not only a biomarker, but also, more importantly, an active promoter of inflammatory disease processes in that they stimulate endothelial cells, PMNs, and notably also platelets. The latter was demonstrated in experiments in which PMPs were stimulated by soluble phospholipase A2: the newly formed AA is then rapidly converted by PMPs to TXA2, a classic activator of platelets (14). In line with this view, ExoU, a Pseudomonas aeruginosa cytotoxin with phospholipase A2 activity, increases both PMP release and TXA2 formation and accordingly resulted in lung thrombus formation and increased vascular permeability in a murine model of pneumosepsis (101). Overall, a series of proinflammatory effects of PMPs has emerged that is based on the activation of platelets, monocytes, and vascular endothelial cells either directly by MP-derived AA or by its metabolism to bioactive prostanoids (12, 13, 15). This recognition substantiates a critical role of PMPs in ALI, since TXA2 contributes pivotally to lung vascular leakage and inflammation in experimental models of the disease (72).
Platelet-PMN and endothelial-PMN costimulation may not only be promoted by PMPs, but likewise by PMN microparticles (NMPs), since endotoxin (LPS) can stimulate adherent PMNs to release NMPs that generate platelet-activating factor (PAF), thus causing activation of platelets (172), endothelium (149), and other inflammatory cells. In the air spaces, NMPs may also trigger inflammatory responses and epithelial cell damage, as suggested from their abundance in sputum from patients with cystic fibrosis (136). NMPs can furthermore directly activate endothelial cells to generate proinflammatory cytokines such as IL-1β, interleukin 1β; INFγ, interferon-γ; LMP, lymphocyte MP (red); LPS, lipopolysaccharide; LXA₄, lipoxin A₄; MacMP, macrophage MP (pink); MMP, monocyte MP (purple); MP, microparticle; NMP, polymorphonuclear neutrophil MP (orange); PAI-1, plasminogen activator inhibitor 1; PHA, phytohemagglutinin; PMN, polymorphonuclear neutrophil; PMP, platelet MP (light green); RMP, red blood cell MP (dark green); ROS, reactive oxygen species; TFMP, tissue factor expressing microparticle (dark purple); TNF-α, tumor necrosis factor-α; TXA₂, thromboxane A₂.

Lastly, inflammatory diseases such as ALI and ARDS are often associated with immune modulation and its complex functional consequences including inflammatory damage to bystander cells (123, 158, 173). Such immune modulation also involves platelets that can actively alter innate and adaptive immune function (94, 152). For example, PMPs can stimulate endothelial cells to generate a range of proinflammatory cytokines including IL-1, IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) as well as monocyte-derived IL-1, TNF-α, and IL-8 (reviewed in Refs. 20, 120). MPs have also been directly associated with immunomodulatory effector molecules such as RMP- and PMP-derived CD40L, IgG, and complement found in stored blood products (80). Immunomodulatory and proinflammatory effects of MPs may be particularly relevant in the context of transfusion-related ALI (TRALI), because storage of blood products will propel for-
formation of MPs (25, 33, 144) that will subsequently activate PMNs (80). Taken together, MPs of diverse cellular origins including LMPs, PMPs, RMPs, NMPs, and EMPs directly or indirectly promote inflammatory responses and may thus propel ALI, in that they stimulate cytokine release and promote PMN activation, migration, and PMN-platelet interaction.

Coagulation. Severe infections lead to activation of the coagulation cascade and altered fibrinolysis, with the lung being a key recipient of fibrin and clotting in ALI and sepsis (165). The hypercoagulable and hypofibrinolytic changes seen in ALI/ARDS contribute pivotally to the pathology of the disease (170, 171) and involve characteristic changes in mediators of coagulation and fibrinolysis, including TF, thrombomodulin, TFPI, PAI-1, PS, and von Willebrand factor (vWF).

TF is considered to play an important role in the pathophysiology of lung injury, because it can activate PAR1 and PAR2, which are involved in inflammatory and procoagulatory responses causing fibrin deposition in the lung (82, 165). Importantly, TF is expressed not only on activated mononuclear cells, endothelial cells, alveolar epithelium, or respiratory macrophages, but likewise on many MPs (11, 165). The procoagulant activity of PMP membranes exceeds that of their parent platelet membranes by up to 100-fold (116, 156). In vascular homeostasis, the effects of TF are counteracted by thrombomodulin, an endothelium-derived anticoagulant factor, thus increasing thrombogenicity in vitro (4), whereas TF is expressed not only on activated mononuclear cells, but likewise on many MPs (11, 165). The procoagulant activity of PMP membranes exceeds that of their parent platelet membranes by up to 100-fold (116, 156). In vascular homeostasis, the effects of TF are counteracted by thrombomodulin, an endothelium-derived anticoagulant factor, blocking the TF-induced stimulation of the coagulation cascade by reversibly inhibiting factor Xa and thrombin. Severe infections such as sepsis that constitute typical predispositions for ALI are characterized by an imbalance between elevated TF and lower TFPI levels causing enhanced coagulation and inflammation (165). The relevance of this imbalance is highlighted by observations from humans (56) and experimental models of ALI in which blockade of TF by antibodies (175) or TFPI (48) reduced lung damage and improved pulmonary function. MMPs can attenuate the expression of TFPI and thrombomodulin, an endothelium-derived anticoagulant factor, thus increasing thrombogenicity in vitro (4), whereas TF is abundantly expressed on MPs under inflammatory conditions (91). In healthy human volunteers, MP-borne TF procoagulant activity increased eightfold within 4 h after infusion of LPS (11). Hence, infectious or inflammatory stressors may contribute to and/or aggravate ALI in part by the formation and release of TF-studded MPs that in the presence of an attenuated anticoagulatory response will generate the characteristic prothrombotic milieu associated with ALI, especially in consideration of the previously discussed fact that TF on MPs is often decrypted and active. Notably, TF activity on MPs in ALI/ARDS is not necessarily confined to the vascular compartment (16), but, in line with the clinical presentation of fibrin clots in the distal air spaces, may similarly apply to the alveolar compartment, where increased TF production by alveolar epithelial cells has been documented (18, 165). In pulmonary edema fluid from mechanically ventilated (<7 ml/kg tidal volume) patients with ARDS, Bastarache and colleagues (16) detected higher concentrations of epithelial cell marker receptor for advanced glycation end products (RAGE) and TF-enriched MPs in bronchoalveolar lavage fluid (BALF) compared with patients with hydrostatic lung edema whose MPs also expressed less TF and RAGE. It is possible that ARDS leads to either larger MPs that express more RAGE receptors or perhaps a numeric increase in RAGE-positive MPs compared with ARDS-negative hydrostatic edema controls. Patients who died had a trend toward a higher MP concentration in lung edema fluid compared with those who survived (P = 0.073), attesting to a detrimental effect of MPs in ALI/ARDS. Similar MPs could be generated in vitro by stimulation of cultured alveolar epithelial cells with a mix of the proinflammatory mediators TNF-α, IL-1β, and interferon-γ (16, 17). Whereas both ARDS and non-ARDS controls in Bastarache’s study were severely sick, BALF analyses from healthy pigs with volume-controlled ventilation set to maintain normocapnia (37.5–41 mmHg Paco₂) with 5 cmH2O positive end-expiratory pressure revealed PMPs that were positive for fibrinogen, vWF, and P-selectin (124). This finding gains relevance in light of the fact that mechanical ventilation, in particular at tidal volumes >6 ml/kg body wt, will exacerbate ALI and promote ventilator-induced lung injury. PMPs in BALF were evident after only 1 h of mechanical ventilation, yet, unfortunately, actual tidal volumes applied were not reported in this study so that it is unclear whether this effect was attributable to overventilation or occurs even under conditions of protective ventilation. Notably, presence of PMPs was likewise detected in human tracheal mucus obtained from five humans at extubation after surgery, suggesting that this finding may reflect a more general effect of mechanical ventilation and/or surgical trauma.

Similar to TF expressing MPs, a procoagulant environment in ALI may be likewise promoted by PMPs. However, the degree to which circulating PMPs are actually elevated in patients with ALI or ARDS remains to be elucidated. Earlier work by George and coworkers (60) did not detect an increase in blood PMPs in patients with ARDS from various causes, whereas postcardiac bypass patients exhibited an increased plasma concentration of PMPs. Yet it is conceivable that the radiolabeled GPIIb antibody technique used to assay PMPs in this study did not screen for the entire population of PMPs in the ARDS cohort. Beyond TF expression, MPs expose large amounts of PS on their outer membranes, thus creating an ideal surface for activation of the coagulation pathway (148). In addition, MPs generate and release other prothrombotic factors including thrombin (80) or vWF multimers that can promote and stabilize platelet aggregation (20). In summary, there is ample evidence from in vitro animal and human studies demonstrating the formation of MPs with procoagulant activity under inflammatory conditions and in ALI in both the vascular and alveolar compartment, where they are likely to contribute pivotally to the hypercoagulable and hypofibrinolytic state characteristic for ALI/ARDS.

Permeability. The increased permeability of capillary and alveolar barriers in ALI leads to the extravasation of high molecular weight protein and inflammatory cells into the air spaces of the lung, thus impairing respiratory mechanics, attenuating gas exchange, causing increased shunting and ventilation perfusion mismatch, and contributing to the consolidation of lung tissue during the subsequent fibroproliferative phase of ALI/ARDS. Several biolipid mediators such as TXA2, phospholipids, and sphingolipids have been implicated in the pathogenesis of permeability-type lung edema and are notably also linked directly or indirectly to the formation and function of MPs (62, 104).

As discussed before, PMPs can promote the formation of TXA2 from AA (14). This finding gains relevance in the context of permeability-type edema because TXA2 inhibitors
can reduce lung vascular permeability in animal models of ALI (72, 182). In line with this view, increased levels of circulating PMPs were associated with elevated TXA2 plasma concentrations and lung vascular permeability in a mouse model of bacterial pneumonia with histological evidence of thrombus formation in the lungs (101). In sepsis, the increased MP-associated formation of TXA2 may serve to maintain a basal vasopressor effect (120, 122), yet this beneficial effect does not translate to the low pressure system of the lung where increased TXA2 formation will primarily promote lung edema.

In addition to eicosanoids, phospho- and sphingolipid mediators such as PAF and ceramide have been proposed to contribute critically to vascular hyperpermeability in ALI (62). PAF can be expressed on the surface of NMPs and promote platelet aggregation and association with PMNs (172), whereas ceramide has been implicated in MP formation due to its involvement in lipid rafts (155). Acid sphingomyelinase (ASM), which catalyzes the conversion of sphingomyelin to ceramide, triggers the release of brain glial MPs, an effect that is blocked by ASM inhibitors or in astrocytes obtained from ASM-deficient (Smpd1−/−) mice (23). Although it remains to be shown whether a similar ASM-mediated mechanism contributes to MP formation in other nonglial cell types, this finding raises the intriguing notion that the demonstrated detrimental effects of ASM/ceramide signaling in ALI (87) relate to the increased formation of MPs.

Endothelial function. Apart from biolipid mediators, various MPs may directly impact endothelial function, thereby critically regulating vascular barrier properties as well as promoting the hyperinflammatory and hypercoagulatory responses discussed in previous sections. EMPs can attenuate NO release from cultured endothelial cells and induce a corresponding decrease in eNOS phosphorylation at Ser1179 and a decrease in hsp90 association consistent with the notion of EMP-induced endothelial dysfunction (44). In line with this view, EMP treatment impaired endothelium-mediated vasodilation in both mouse and human ex vivo vessel preparations (44). Likewise, EMPs attenuate vasorelaxation in rat aortas in a dose-dependent fashion involving impaired NO signaling and endothelial function (29). These findings gain relevance in the context of ALI in view of the frequently barrier-protective (85, 179), anti-inflammatory, and anticoagulatory effects of basal endothelial NO production in the lung. Endothelial dysfunction and impaired aortic vasorelaxation were likewise inducible in mice by LMPs, which caused overexpression of caveolin-1 and concomitant inhibition of eNOS (106). Endothelial NO production is also inhibited by smooth muscle cell MPs via a β3-integrin-mediated mechanism (49). The relevance of impaired endothelial NO formation in the context of ALI and inflammation is highlighted by the fact that IL-8-dependent PMN migration and endothelial transmigration in vitro are enhanced by the NO synthase inhibitor N3-nitro-L-arginine methyl ester (L-NAME), and likewise by NMPs generated by L-NAME treatment, but not by d-NAME or untreated PMNs (125). Conversely, NO may also regulate MP formation although in different ways depending on cell type and/or experimental conditions, as highlighted by the finding that L-NAME induced NMP formation from PMNs (125), whereas macrophage MP formation in response to Toll-like receptor agonists such as LPS is reduced by inhibition of inducible nitric oxide synthase (iNOS) (59). Taken together, MP formation can be modulated by the bioavailability of NO, and once formed MPs can attenuate local NO production, in particular with respect to endothelial cells (20), which in turn may promote ALI and barrier permeability. Accordingly, EMPs derived from PAI-1-stimulated endothelial cells can induce direct ALI in mice and stimulate the release of IL-1β and TNF-α, leading to increased PMN recruitment to the lung (31). In Brown Norway rats, intravenous infusion of EMPs at pathophysiologically relevant concentrations induced characteristic signs of pulmonary edema, lung PMN infiltration, and increased lung vascular permeability (44). Increased lung capillary permeability was likewise evident in C57BL/6 mice when EMPs were infused as either primary or secondary hit (44). EMPs infusion into C57BL/6 mice likewise led to increased inflammatory cytokine release and lung PMN recruitment (31) and exacerbated LPS-induced lung injury. Notably, the aggravation of LPS-induced lung injury was particularly prominent when EMPs were infused prior to rather than simultaneous with LPS (31), indicating that EMPs can both prime the lung for subsequent injurious stimuli or exacerbate lung damage in previously challenged lung tissue.

Clinical implications. The recognition of a potential detrimental effect of MPs in the pathophysiology of ALI/ARDS opens up new therapeutic perspectives, in that removal of MPs and/or inhibition of MP functions may present promising strategies for future treatment interventions (32, 184). Based on a similar rationale, removal of PMPs by a membrane plasma separator was recently tested in eight patients undergoing apheresis, yet although considerable amounts of PMPs were removed by filtration total numbers of circulating PMPs could not be diminished by this intervention, potentially because of a stimulation of MP formation by the plasmapheresis procedure per se (66). Compared with mechanical removal, pharmacological attenuation of circulating MP levels may not only be feasible but may have inadvertently already been introduced into the treatment of inflammatory diseases, since glucocorticoid treatment has been shown to significantly reduce circulating PMP levels in patients with polymyositis and dermatomyositis (154). Yet at the current stage the significance of immunosuppression or immunomodulation by glucocorticoids in the treatment of ALI/ARDS remains unclear owing to mixed clinical results (173), and a thorough analysis of the effects of steroid administration on circulating MP levels and subpopulations is yet lacking.

Notably, agonists of peroxisome proliferator-activated receptor (PPAR)-γ such as rosiglitazone, which have been introduced as antidiabetic drugs but also show efficiency in lung diseases such as pulmonary hypertension (67, 68), may present an alternative approach to target deleterious MP functions in inflammatory diseases. MMPs increase macrophage and monocyte superoxide anion production, cytokine release, and NF-κB activation via a PPAR-γ-regulated mechanism that can be inhibited by PPAR-γ agonists (5). PPAR-γ agonists have also been demonstrated to inhibit LMP-mediated vascular dysfunction and increased release of proinflammatory proteins from isolated murine aortae (120) and the upregulation of proinflammatory cytokines including IL-8 and monocyte chemotactic protein-1 by monocyte MPs in cultured human lung epithelial cells (83). PPAR-γ expression has been shown to be reduced in ALI (97) whereas agonists of PPAR-α or -γ can attenuate experimental ALI (9, 98, 150). Although PPAR agonists may...
thus have therapeutic potential in the context of ALI/ARDS, their effects on circulating MP levels and MP function in this disease remain to be determined.

Several reviews of MPs have highlighted the interesting strategy to modulate PMP formation by statin or polyunsaturated fatty acid (PUFA) therapy (42, 91). Although PUFAs have been pursued as therapeutic interventions in ARDS with reported benefits in terms of decreased permeability and PMN recruitment (157), statin treatment remains controversial yet is actively pursued as potential therapy for ALI/ARDS (26, 86). Because randomized clinical trials are currently underway, it will be of interest to see whether potential benefits of statin therapy in ARDS are likewise associated with reduced levels or altered profiles of circulating MPs.

Importantly, attenuation of MP formation may also provide an attractive strategy to reduce complications associated with the administration of stored blood products, including TRALI. RMPs typically form after 10 days of storage, whereas PMPs accumulate earlier within days, and levels of both can be significantly reduced if packed cells are leukoreduced or in the presence of storage additives such as glucose, pyruvate, inosine, adenine, and phosphate (80). Storage of platelets with PGE$_2$, theophylline, or aprotinin as well as pharmaceutical inhibition of α$_{III}$β$_{3}$-integrin signaling have been proposed to reduce ex vivo PMP production (25, 33) and may thus help to reduce PMN activation and sequestration in the lungs subsequent to transfusion of stored blood products. Compounds such as cyclosporin A and Orai 1 inhibitors have been proposed as a means of decreasing levels of EMPs and PMPs, respectively (5, 10, 21, 32). Further work is required to examine the effects of eliminating or altering selected MP populations generated in stored blood products to test whether such interventions may positively impact the mortality and morbidity of transfused experimental animals and ultimately patients.

Potential Beneficial Effects of Microparticles in ALI

MPs are not necessarily always associated with progressive organ dysfunction and disease outcome. As recently discussed in seminal reviews by Morel and coworkers (120) and Dignat-George and Boulanger (46), MPs are by no means always detrimental but may frequently exert beneficial effects, in that they exhibit anti-inflammatory and anticoagulant potential and can improve endothelial and vascular function under pathological conditions (Fig. 7). Experimental evidence for the beneficial effects of MPs comprise the promotion of myocardial tissue repair postinfection by PMPs, the stimulation of matrix degradation and new blood vessel formation by EMPs through activation of matrix metalloproteases, or the triggering of angiogenic endothelial responses and the reconstitution of endothelial function and NO generation associated with reduced ROS production by EMPs carrying the developmental morphogen sonic hedgehog (3, 20, 114). These findings exemplify the seemingly paradoxical beneficial effects that have been documented for a variety of MPs under different pathological conditions, a notion that gains particular relevance in the context of ALI by the clinical observation that higher levels of circulating LMPs or EMPs, respectively, are associated with increased survival in both sepsis and ARDS (65, 158). The potential role of beneficial MPs gains additional momentum in light of the fact that considerable numbers of MPs can also be released by mesenchymal stem cells (MSCs) and may thus, at least in part, account for the paracrine effects by which MSCs have emerged as a promising new therapeutic strategy in ALI (63, 92, 93, 107). MSCs secrete MPs that are enriched in pre-miRNAs (35) and, in a rat renal injury model, could inhibit apoptosis and promote proliferation of kidney epithelial cells by paracrine effects that are in part mediated via intercellular transfer of mRNA and miRNA (58). MSCs have also recently been shown to donate mitochondria to lung parenchymal cells in ALI by a microvesicular mechanism that could imply MPs (73); yet the relevance of mitochondrial transfer for the effects of MSCs has been challenged by others (38). Further investigations are needed to characterize the contributions of MSC-derived MPs in the benefits these cells appear to offer in the setting of lung injury. In the following, we will discuss mechanisms that may potentially contribute to the beneficial effects of non-stem-cell-derived MPs in ALI focusing on the same pathophysiologial characteristics of the disease as previously done for the detrimental role of MPs.

Inflammation. Although we previously emphasized the role of TXA$_2$ in the context of the delivery and release of AA and AA metabolites by MPs, it must be taken into consideration that not all eicosanoids are proinflammatory and increase barrier permeability. Notably, PMPs contain lipooxygenase 12 that, when transferred to mast cells, promotes production of the anti-inflammatory leukotriene lipoxin A$_4$ (LXA$_4$) (161). In a murine model of experimental colitis, injection of PMPs and LXA4 attenuated inflammatory responses, whereas lipooxygenase 12 protein-deficient MPs or mast cell depletion reversed this beneficial effect (161). Since LXA4 analogs have been shown to attenuate ALI in animal models (47, 76), MP-mediated generation of LXA4 might prove beneficial in ALI.

Although certain MPs formed in ALI may have the ability to cause detrimental immune modulation as described in previous sections, others may confer beneficial immune functions capable of enhancing survival or reducing morbidity. NMPs can stimulate secretion of transforming growth factor-β, which blocks macrophage activity, and can express annexin A1,
which acts as an anti-inflammatory protein blocking adhesion of PMNs to endothelial cells (40, 57). In addition, MPs generated from KATO-III human gastric cancer cells can interact with monocytes and change their cytokine release profile from proinflammatory mediators such as TNF-α to anti-inflammatory cytokines such as IL-10 (83). Although the relevance of such effects in the context of ALI/ARDS remains to be elucidated, these findings underscore the anti-inflammatory potential harbored by many MPs. A recent analysis of ARDS patients demonstrated elevations in both LMPs and NMPs within BAL samples of ARDS patients compared with spontaneously breathing controls (65), whereas levels of LMPs did not differ among ARDS patients compared with simplified acute physiology score (SAPS II) and sequential organ failure assessment (SOFA) score matched ventilated intensive care patient controls without ARDS and spontaneously breathing patients being bronchoscopically investigated for suspected chronic lung diseases. Notably, survivors of ARDS had statistically elevated levels of LMPs compared with the ventilated and spontaneously breathing control groups. This unexpected finding raises the possibility that LMPs may not only be a biomarker of ARDS and/or potentially promote inflammatory signaling and parenchymal damage but may at times also be protective and reflect a “healthy” level of immune function. This physiological effect may be absent in severe inflammatory diseases such as ARDS, causing “immune paralysis” or a relative anergy of immune function that may have ramifications on the host that directly impact mortality (123).

**Coagulation.** Whereas the high expression levels of TF in MPs are generally considered to be procoagulant and barrier-disruptive, they may also have anticoagulant and barrier-protective properties through PAR1-mediated transactivation of PAR2 signaling pathways (82). In addition, MPs can express a variety of anticoagulant factors including thrombomodulin, TFPI, endothelial protein C receptor (EPCR), and protein S (120). Thus the potential involvement of MPs in anticoagulant pathways may present both an intrinsic protective mechanism and a possible future therapeutic target in lung injury. Notably, LMPs from hepatitis C patients have been shown to cause increased fibrinolysis after fusion with hepatic stellate cells, thus decreasing fibrin deposition (84).

Activated protein C (APC) proteolytically inactivates key coagulation factors including factor Va and factor VIIIa. Recombinant APC has been shown to reduce ALI and increase alveolar ventilation in experimental animal models (71, 110, 167) and individual clinical case reports (81). In sepsis patients, treatment with APC has been reported to show a trend toward increased survival that is notably associated with MP formation and concomitant anti-inflammatory effects (132). However, a recent Cochrane review did not recommend its use because of a failure to improve survival and an increased bleeding risk, which has led to a decrease in its use as a therapy for severe sepsis (105). Interestingly nevertheless in the context of this review, APC induces the release of MP-associated EPCR that can bind APC and exert anticoagulatory (131) and potentially also anti-inflammatory and barrier-protective effects (120). EPCR (131) is a member of the major histocompatibility complex/CD1 superfamily that promotes protein C activation at the cell surface (159), yet upon metalloprotease-mediated cleavage circulates in a soluble form (sEPCR) (88) that neutralizes APC so that it can no longer inactivate factor Va (95). Importantly, APC bound to EPCR in microparticulate form retains anticoagulant activity (131), and has been shown to exert both antiapoptotic and barrier-protective effects (130).

**Permeability.** APC may also exert important barrier-protective effects, in that EPCR ligation promotes transactivation of sphingosine 1-phosphate receptor 1 (S1P1) via phosphatidylinositol 3-kinase (PI3K)-dependent phosphorylation (51). Hence, APC-bearing MPs may limit vascular permeability via the well-documented barrier-enhancing signaling cascade downstream of S1P1 (169). This notion is supported by experimental data demonstrating that EPCR/APC-bearing EMPs reduce endothelial permeability in vitro via a mechanism that involves PAR-1, PI3K, and notably also the vascular endothelial growth factor receptor 2 (130).

**Endothelial function.** Although MPs are frequently associated with endothelial and vascular dysfunction (vide supra), several studies by the group of Ramarosin Andriantsitohaina and Maria Carmen Martinez (3, 114, 121) have demonstrated that the endothelium may also receive beneficial signals from MPs carrying sonic hedgehog, resulting in correction of endothelial injury, reconstitution of NO release, and reduced ROS production. The role of NO in this context, however, is complex because it can exert both attenuating and aggravating effects on inflammatory, coagulatory, or permeability changes depending on its location, concentrations, and local microenvironment (85). Although a recent meta-analysis did not support the rationale for inhaled NO as an effective treatment strategy in ALI/ARDS (2), reconstitution of endogenous NO production and an intact endothelial and vascular function may nonetheless provide important benefits that could positively impact on clinical outcome in ALI/ARDS.

**Clinical implications.** Taken together, MPs possess a variety of mechanisms by which they may exert anti-inflammatory, anticoagulatory, and barrier-enhancing effects under healthy or diseased conditions. Although our understanding of these properties of MPs in general, and in the context of ALI/ARDS in particular, is still in its infancy, the potential to exploit this intrinsic property for therapeutic purposes poses a challenging yet promising path to pursue.

**Synopsis and Implications**

There has been little in the way of effective innovations and interventions in the treatment of ALI/ARDS beyond the introduction of protective ventilation (1) and restrictive fluid management strategies (176). ALI/ARDS is commonly associated with increased formation of MPs from various cellular origins found in both the distal air spaces and circulating blood. These MPs are small vesicles with an intact lipid bilayer that may contain membrane and cytosolic proteins, lipids, organelles, and genetic material from their parent cells that can function autonomously or transfer their contents to other cells. Although most studies have looked at MPs as potential biomarkers for disease and progression of illness, MPs bear the potential to convey both detrimental as well as beneficial effects with respect to the excessive inflammatory, coagulatory, and permeability responses characteristic for ALI/ARDS (Fig. 6). Further investigations into MPs’ actions are required to better understand their role in the pathophysiology of acute lung disease and to utilize their potential for therapeutic interventions. Such concepts may include strategies to prevent the
formation of MPs by altering flip-flop scramblase enzyme activities (116) or therapeutic manipulation of MP populations by pharmacological interventions (138). Even more attractive is the potential perspective to use specifically engineered “designer” MPs as vectors to move genes, proteins, or specific cellular functions between cells (20). Clearly, MPs add another layer of complexity to the already multifaceted mechanisms involved in ALI/ARDS, but in parallel they may offer hope of obtaining and specific sites for future pharmacological manipulation.

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REFERENCES


MICROPARTICLES AND ACUTE LUNG INJURY

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