Perspective

Neutrophil Intercellular Communication in Acute Lung Injury

Emerging Roles of Microparticles and Gap Junctions

Viola Dengler[1], Gregory P. Downey[2,4], Rubin M. Tuder[3], Holger K. Eltzschig[1], and Eric P. Schmidt[3]

[1]Department of Anesthesiology, [2]Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine and Integrated Department of Immunology, and [3]Program in Translational Lung Research, Department of Medicine, University of Colorado School of Medicine, Aurora, Colorado; and [4]Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Medicine, National Jewish Health, Denver, Colorado

A hallmark of acute inflammation involves the recruitment of polymorphonuclear leukocytes (neutrophils) to infected or injured tissues. The processes underlying this recruitment are complex, and include multiple mechanisms of intercellular communication between neutrophils and the inflamed tissue. In recent studies of the systemic and pulmonary vasculature, interest has increased in novel forms of intercellular communication, such as microparticle exchange and gap junctional intercellular communication. To understand the roles of these novel forms of communication in the onset, progression, and resolution of inflammatory lung injury (such as acute respiratory distress syndrome), we review the literature concerning the contributions of microparticle exchange and gap junctional intercellular communication to neutrophil–alveolar crosstalk during pulmonary inflammation. By focusing on these cell–cell communications, we aim to demonstrate significant gaps of knowledge and identify areas of considerable need for further investigations of the processes of acute lung inflammation.

Keywords: neutrophil; connexin; gap junction; microparticles; acute lung injury

Although investigations of acute respiratory distress syndrome (ARDS) have yet to yield an efficacious pathophysiology-targeted therapy, they have provided important insights into the intercellular communications regulating neutrophil activation and alveolar transmigration. These communications include juxtacrine and paracrine (e.g., chemokines, cytokines, and proteinases), crosstalk between neutrophils and lung parenchymal cells, and the signaling conducted through cell-surface receptors such as leukocyte integrins and cognate adhesion molecules (e.g., intercellular adhesion molecules) expressed by lung parenchymal cells (1). The past decade has seen an increasing recognition of alternative forms of intercellular communications during the onset and resolution of inflammatory lung disease. These communications include microparticle exchange as well as gap junctional intercellular communication (GJIC). This Perspective will focus on these alternative forms of communication between neutrophils and lung parenchymal cells during the genesis of ARDS, highlighting opportunities for further investigations.

CLINICAL RELEVANCE

The acute respiratory distress syndrome (ARDS) is a common yet highly morbid critical illness. Despite more than 40 years of investigation, no effective disease process–targeted treatment for ARDS exists. By highlighting promising new mechanisms of cell–cell communications in ARDS, we hope to identify new avenues of research that could yield novel therapeutic approaches.

MICROPARTICLE EXCHANGE

Microparticles are spherical, lipid bilayer–encapsulated extracellular bodies ranging from 50–1,000 nm in diameter. Microparticles can be secreted by almost every cell type, including lung parenchymal cells and inflammatory cells. Multiple forms of microparticles exist, reflecting different modes of production. "Exosomes" and "shedding vesicles" are derived from living cells, whereas "apoptotic bodies" are secreted by apoptotic and/or necrotic cells (2). Exosomes have an endosomal origin, and are stored as intraluminal vesicles within multivesicular bodies (3). Upon stimulation, exosomes are secreted by fusion with the cell membrane, forming a relatively homogenous group of microparticle sizes (50–150 nm). In contrast, shedding vesicles and apoptotic bodies derive from the budding of small cytoplasmic protrusions from the plasma membrane (4). These represent a more heterogeneous group of membrane vesicles (50–1,000 nm).

Although the microparticle release rate in resting cells is very low, the secretion of shedding vesicles can be induced by various inflammatory stimuli associated with altered intracellular calcium concentrations (5, 6). In contrast, exosome secretion can occur independently of any calcium influx, and may happen spontaneously (7, 8). Released microparticles may be ultimately internalized by local or distant recipient cells via mechanisms ranging from specific receptor–ligand interactions (indicating a targeted, cell-specific delivery) to nonspecific internalization via endocytosis or simple fusion with the cell membrane (9–12). Microparticles may therefore serve as a circulating "storage pool" of bioactive effectors (13) that mediate intercellular crosstalk and the horizontal transfer of genetic material, including microRNAs (miRNA) (14). As such, microparticles may participate in biological functions (including

(*Received in original form November 20, 2012 and in final form January 23, 2013*)

This work was funded by National Institutes of Health and National Heart, Lung, and Blood Institute grants K08-HL105538 (E.P.S.), R01-ES0162 (R.M.T.), R01-HL0921, R01-DK083385, and R01-HL098294 (H.K.E.), and R01-HL090669 (G.P.D.).

Author Contributions: V.D. and E.P.S. jointly wrote the manuscript. G.P.D., R.M.T., and H.K.E. contributed to the conceptual framework and edited the manuscript.

Correspondence and requests for reprints should be addressed to Eric P. Schmidt, M.D., Program in Translational Lung Research, Department of Medicine, University of Colorado School of Medicine, 12700 East 19th Avenue, Research Complex 2, Mail Stop C272, Aurora, CO 80045. E-mail: eric.schmidt@ucdenver.edu

Am J Respir Cell Mol Biol Vol 49, Iss. 1, pp 1–5, Jul 2013

Originally Published in Press as DOI: 10.1165/rcmb.2012-0472TR on March 14, 2013
Internet address: www.atsjournals.org

hemostasis, cell activation, and inflammation) relevant to the pathogenesis of ARDS (15).

GAP JUNCTIONAL INTERCELLULAR COMMUNICATION

In contrast to the distant interactions enabled by microparticle exchange, gap junctions allow for contiguous cell–cell communication. Gap junctions are formed from cell-surface connexins, a 20-member family of transmembrane proteins (16). Connexins associate into hexagonal connexons, which constitute 2–3-nm-diameter pores permeable to small (< 1 kD) molecules. A connexon may exist in an unpaired state ("hemichannel") or pair with the connexon of a neighboring cell, forming a gap junction capable of direct cytoplasmic exchange. Recent studies have demonstrated the importance of gap junctional intercellular communication (GJIC) to the onset (17) and resolution (18) of pulmonary inflammation.

RELEVANCE OF ALTERNATIVE COMMUNICATION MECHANISMS TO NEUTROPHIL FUNCTION

The physiological significance of microparticle exchange and GJIC has been largely demonstrated in cell types other than neutrophils. However, available evidence supports the relevance of these processes to neutrophil function during inflammation.

Neutrophils rapidly shed microparticles in response to inflammatory stimuli (Figure 1) (19, 20). Shed neutrophil microparticles are heterogeneous, ranging from 50–200 nm in size (21). Neutrophil microparticles typically display surface phosphatidylserine as well as neutrophil markers such as selectins, CD15, CD64, CD66b, and CD66e (20, 22). As will be described, neutrophil-derived microparticle exchange exerts a significant impact on processes germane to organ inflammation, including inflammatory cell adhesion, cytokine production, and chemotaxis.

In contrast to microparticle exchange, the participation of neutrophils in GJIC is less well understood. Activated neutrophils express transmembrane connexins, including connexins 43 (23, 24) and 40 (25, 26). These connexins are capable of forming cell-surface connexons, which, if unpaired, function as channels for the extracellular secretion of small molecules such as ATP (24). Should neutrophil connexons link with the connexons of an adjacent cell, GJIC may occur. Indeed, neutrophil–neutrophil (25) and neutrophil–endothelial (23, 26) GJIC has been documented.

DO THESE COMMUNICATIONS CONTRIBUTE TO ARDS?

Although microparticle exchange and GJIC may be of relevance to neutrophil physiology, their contributions to the pathogenesis of ARDS remain under investigation. We will discuss studies examining the activated neutrophil at several key stages in the evolution of lung inflammation: (*1*) the activated, circulating neutrophil, (*2*) the endothelial-adherent neutrophil, (*3*) the transmigrating neutrophil within the interstitial space, and (*4*) the activated neutrophil within the alveolar space.

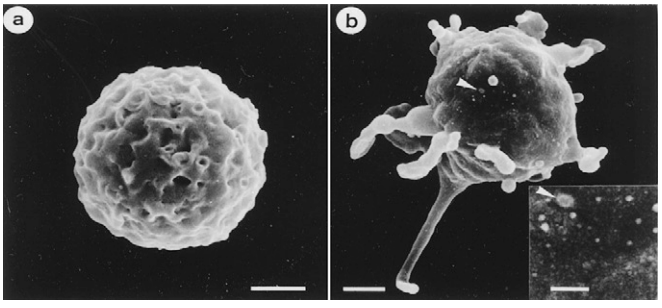

Figure 1. Microparticle release from activated neutrophils. Scanning electron micrograph of inactive (*a*) and FMLP-activated (*b*) neutrophils. *Arrowheads* (in *main image* and *inset*) demonstrate a 150-nm-diameter vesicle budding from the neutrophil surface, consistent with microparticle release. *Scale bar*, 1.5 μm (400 nm on *high-power inset*). Reproduced with permission from Ref. 19.

Circulating Neutrophils during Systemic Inflammation

Systemic inflammatory diseases are characterized by rapid changes in circulating neutrophil morphology and physiology (27), thought to be mediated by the circulating cytokines and chemokines characteristic of the systemic inflammatory response syndrome (SIRS). The role of alternative forms of intercellular communication is less clear.

Neutrophil interactions with non-neutrophil microparticles. Neutrophils are known to interact with circulating microparticles of nonleukocyte origin (28–30). Opportunities for neutrophil–microparticle interaction are increased during SIRS, given a significant increase in circulating endothelial and platelet microparticles (28, 31).

Septic shock is associated with a significant increase in circulating platelet microparticles (31), with evidence of increased microparticle–neutrophil interactions (30). These complexes, although linked to improved patient outcomes, are also associated with augmented leukocyte activation (30). Indeed, platelet microparticles can activate neutrophils (29, 32, 33) and, in doing so, facilitate the progression of intravascular coagulation (34).

Similarly, circulating endothelial microparticles are more abundant in patients with sepsis (31), and complex with circulating leukocytes (28). Monocyte–endothelial microparticle complexes are associated with improved patient outcomes in sepsis (30). Although neutrophil–endothelial microparticle interactions have been similarly observed in patients with SIRS (28), the physiological impact of these interactions is largely unexplored.

Circulating erythrocyte microparticles may additionally activate neutrophils during ARDS (35), a finding of particular interest to the pathogenesis of transfusion-related acute lung injury (TRALI).

Neutrophil production of microparticles. Neutrophils not only receive communications via microparticle exchange, but also produce microparticles, influencing the function of several cell types relevant to lung injury (Table 1). Consistent with these diverse effects, the overall clinical impact of neutrophil-derived microparticles is uncertain. Circulating leukocyte and erythrocyte microparticles isolated from septic rats induced hemodynamic instability in normal rats (43). In contrast, nonplatelet microparticles isolated from humans with sepsis improved the vasoconstrictor response of murine aortic rings (31). Indeed, high circulating concentrations of leukocyte-derived microparticles were associated with improved outcomes in ARDS (44).

Direct cell–cell contact between neutrophils and other circulating cells. Intravascular neutrophils may directly interact with other circulating cells during systemic inflammatory responses. Neutrophils form clusters in response to inflammatory stimuli, with evidence of homotypic neutrophil GJIC (25). Neutrophils may additionally complex with platelets during sepsis (45). Because platelets are capable of forming functional connexons (46, 47), these complexes may allow for neutrophil–platelet GJIC. Interestingly, the presence of circulating platelet–leukocyte conjugates was associated with improved outcomes in septic shock (30). Whether this benefit indicates a protective effect of circulating platelet–leukocyte complexes, or rather reflects a harmful effect of their tissue sequestration, remains uncertain (45, 48). Indeed, platelet–neutrophil complexes release extracellular DNA, forming neutrophil extracellular traps implicated in TRALI (49). The presence and relevance of platelet–neutrophil GJIC in these processes are unexplored.

TABLE 1. EFFECTS OF NEUTROPHIL-DERIVED MICROPARTICLES ON CELL TARGETS IN ARDS

Cell Target	Effect	Mechanism	Reference
Endothelial cells	Proinflammatory	Increased IL-6, MCP-1, tissue factor expression, and JNK-1 activation	(36, 37)
Neutrophils	Anti-inflammatory	Delivery of annexin A1 to neutrophil receptor ALX	(38)
Macrophages	Anti-inflammatory	Increased TGF-β release; decreased IL-8, IL-10, and TNF-α release; down-regulation of TLR2 expression	(11, 39, 40)
Platelets	Prothrombotic	Activated platelet Akt signaling via integrin and selectin binding; delivery of platelet-activating factor	(41, 42)

Definition of abbreviations: ARDS, acute respiratory distress syndrome; JNK-1, c-Jun N-terminal protein kinase–1; MCP-1, monocyte chemoattractant protein–1; TGF-β, transforming growth factor–β; TLR2, Toll-like receptor–2.

Neutrophils Adherent to the Vascular Endothelial Surface

Adherent neutrophils may elaborate circulating microparticles capable of remotely influencing cells relevant to lung injury (as reviewed in Table 1). In addition, adherent neutrophils may participate in intercellular communications that can directly alter the vascular surface as well as the neutrophil itself.

Neutrophil–endothelial interactions at the pulmonary vascular surface. Endothelial cells express connexins (50, 51) capable of forming functional gap junctions with neighboring endothelial cells (17, 52). Cytoplasmic dye transfer experiments and compatible electron microscopy (Figure 2) have also suggested possible endothelial GJIC with recruited neutrophils (23, 26). Surprisingly, inflammatory insults generally attenuate GJIC (26, 53), despite an increase in the connexin content of cells in the distal lung (18, 54). This discordance may arise from the inflammatory internalization of endothelial connexin 43 (50).

The impact of neutrophil–endothelial GJIC (or the inflammatory loss thereof) upon pulmonary inflammation is unclear. The loss of endothelial connexin 43 was associated with an attenuation of leukocyte adhesion in the cremasteric microcirculation (55). Concordantly, proinflammatory effects of pulmonary connexin 43 were observed, although these findings were thought to reflect endothelial–endothelial GJIC (54). Conversely, an *in vitro* study using human umbilical vein endothelial cells reported that gap junction blockade augmented neutrophil transmigration (26).

Microparticle transfer may complement GJIC in mediating neutrophil–endothelial communication at the vascular surface. GJIC between alveolar mesenchymal stem cells (MSCs) and epithelial cells triggers the calcium-mediated release of MSC microparticles, which transfer MSC mitochondria to the alveolar surface (18). A similar pathway could conceivably occur between adherent neutrophils and the pulmonary endothelium, given observations that adherence is a major stimulant to neutrophil microparticle release (42). The adhesion-stimulated release of neutrophil microparticles may either augment neutrophil chemotaxis and endothelial transmigration by coating the endothelial surface with L-selectin and P-selectin glycoprotein ligand–1 (56), or conversely, attenuate inflammation via the delivery of annexin-A1 to recruited neutrophils (38).

Neutrophil–platelet interactions at the pulmonary vascular surface. Neutrophils adherent within the pulmonary circulation may directly interact with platelets. After binding to endothelial cells, neutrophils polarize surface activation of the integrin CD11b/CD18 (macrophage-1 antigen, Mac-1), leading to localized platelet–neutrophil adherence that contributes to inflammatory diseases such as TRALI (57). Given that connexins are expressed in both activated neutrophils and platelets (as already discussed), this polarized binding represents a potential opportunity for GJIC between adherent neutrophils and their platelet partners. The presence and/or functional significance of such GJIC remain unexplored.

Neutrophil Transit through the Pulmonary Interstitium

The first step in neutrophil transit into the interstitium involves the release of the leukocyte trailing edge from the endothelial basolateral surface. In cremasteric microvessels, this release is opposed by neutrophil lymphocyte function–associated antigen 1 (LFA-1)–endothelial intercellular adhesion molecule–1 interactions (58). The leukocyte's attempt to pull away from these adhesions induces the release of $CD18^{+}$ microparticles within the subendothelial space (58). The functional significance of this microparticle deposition, observed during both neutrophil and T-cell transmigration, is unknown, but may serve to direct trailing neutrophils to the site of inflammation.

Despite numerous opportunities for cell–cell interactions during the pulmonary interstitial passage of neutrophils, little else is known about neutrophil GJIC or microparticle exchange during transmigration. Given the propensity of transmigrating neutrophils to approximate pulmonary fibroblasts (59), as well as the ability of fibroblasts to participate in GJIC (60), future investigations of neutrophil–fibroblast interactions may be fruitful.

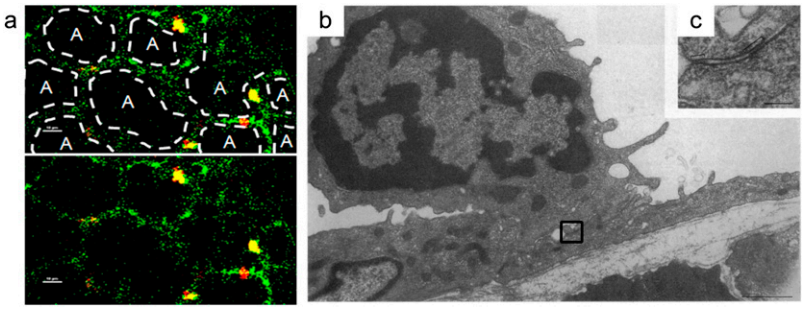

Figure 2. Potential neutrophil–endothelial gap junctional intercellular communication during inflammation. (*a*) Neutrophils exchange cytoplasm with the pulmonary endothelium during endotoxemia. *Top*: Intravital confocal lung microscopy of an LPS-treated (20 μg/g body weight, administered via tail-vein injection), endothelial-fluorescent Tie2–green fluorescent protein (GFP) mouse demonstrates alveoli (A) separated by subpleural microvessels. *Bottom*: Cytoplasmic exchange (*yellow*) occurs between calcein red–labeled polymorphonuclear leukocytes (PMNs) (*red*, PMNs preincubated with 10 μM calcein red × 30 minutes) and endothelial cells (*green*), 30 minutes after application of LPS. *Scale bar* = 10 μm. (*b*) Neutrophil–endothelial cytoplasmic exchange may occur via gap junctions, as suggested by a transmission electron micrograph of neutrophil (located at top of image) adhesion to an endothelial surface within a hamster cheek pouch subjected to ischemia–reperfusion injury. *Scale bar*, 1 μm. (*c*) Enlarged view of selected (*box* in *b*) area demonstrates close membrane apposition (*brackets above cell-cell border* in *c*) consistent with gap junction formation. *Scale bar*, 100 nm. *b* and *c* are reproduced with permission from Ref. 23.

Neutrophils within the Alveolar Space

After transmigration, neutrophils enter into the alveolar space, where they confront invading pathogens and/or cause tissue injury. Despite these important activities, little is known about neutrophil communications occurring within the alveolar space.

Neutrophil–alveolar microparticle exchange. Microparticles detectable within the alveolar space (44, 61) are of varied origin. Epithelial cells have been implicated as a primary source of alveolar microparticles in ARDS (61). Platelet-derived microparticles have been found in porcine bronchoalveolar lavage (BAL) fluid and human tracheal lining fluid (62). Patients with bacterial pneumonia demonstrate increased BAL concentrations of microparticles expressing the neutrophil marker complement receptor–1. These microparticles inhibited the phagocytosis of partly opsonized bacteria *in vitro* (19). The *in vivo* significance of alveolar neutrophil microparticles remains unknown.

Gap junctions. Although epithelial–epithelial gap junctions are of physiologic importance in cystic fibrosis (63), no evidence of neutrophil GJIC with airway epithelia has been observed (64). Alveolar epithelial cells, however, have the potential to form gap junctions with bone marrow–derived cells (MSCs) (18). Whether these processes are relevant to neutrophils remains unknown.

CONCLUSIONS

Although the emerging processes of microparticle exchange and GJIC appear to be of pathophysiologic significance in the inflamed lung, the coordination of these varied intercellular interactions and their overall impact upon lung injury progression are uncertain. However, the pace of scientific discovery regarding microparticle exchange and GJIC is rapid, with multiple recent high-impact publications demonstrating the current enthusiasm for these modes of intercellular communication. Despite these gains, it is particularly striking how little is known about microparticles and/or GJIC after neutrophils exit the vasculature. This gap in knowledge appears to offer fertile ground for future investigations. Improvements in our understanding of neutrophil communication may bring us closer to achieving pathophysiology-targeted therapeutics in ARDS.

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Zemans RL, Colgan SP, Downey GP. Transepithelial migration of neutrophils. *Am J Respir Cell Mol Biol* 2009;40:519–535.
2. Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol* 2009;9:581–593.
3. Février B, Raposo G. Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr Opin Cell Biol* 2004;16:415–421.
4. Beaudoin AR, Grondin G. Shedding of vesicular material from the cell surface of eukaryotic cells: different cellular phenomena. *Biochim Biophys Acta* 1991;1071:203–219.
5. Montoro-García S, Shantsila E, Marín F, Blann A, Lip G. Circulating microparticles: new insights into the biochemical basis of microparticle release and activity. *Basic Res Cardiol* 2011;106:911–923.
6. Morel O, Jesel L, Freyssinet JM, Toti F. Cellular mechanisms underlying the formation of circulating microparticles. *Arterioscler Thromb Vasc Biol* 2011;31:15–26.
7. Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G, Amigorena S. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell–derived exosomes. *Nat Med* 1998;4:594–600.
8. Bhatnagar S, Shinagawa K, Castellino FJ, Schorey JS. Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response *in vitro* and *in vivo*. *Blood* 2007;110:3234–3244.
9. MacKenzie A, Wilson HL, Kiss-Toth E, Dower SK, North RA, Surprenant A. Rapid secretion of interleukin-1β by microvesicle shedding. *Immunity* 2001;15:825–835.
10. De F, El-Assaad F, Mitchell AJ, Alessi MC, Chimini G, Fusai T, Grau GE, Combes VR. Endocytosis and intracellular processing of platelet microparticles by brain endothelial cells. *J Cell Mol Med* 2012;16:1731–1738.
11. Gasser O, Schifferli JA. Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. *Blood* 2004;104:2543–2548.
12. Quesenberry PJ, Aliotta JM. Cellular phenotype switching and microvesicles. *Adv Drug Deliv Rev* 2010;62:1141–1148.
13. Morel O, Toti F, Hugel B, Freyssinet JM. Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. *Curr Opin Hematol* 2004;11:156–164.
14. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of MRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007;9:654–659.
15. McVey M, Tabuchi A, Kuebler WM. Microparticles and acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2012;303:L364–L381.
16. Goodenough DA, Paul DL. Beyond the gap: functions of unpaired connexon channels. *Nat Rev Mol Cell Biol* 2003;4:285–295.
17. Parthasarathi K, Ichimura H, Monma E, Lindert J, Quadri S, Issekutz A, Bhattacharya J. Connexin 43 mediates spread of Ca^{2+}-dependent proinflammatory responses in lung capillaries. *J Clin Invest* 2006;116:2193–2200.
18. Islam MN, Das SR, Emin MT, Wei M, Sun L, Westphalen K, Rowlands DJ, Quadri SK, Bhattacharya S, Bhattacharya J. Mitochondrial transfer from bone-marrow–derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med* 2012;18:759–765.
19. Hess C, Sadallah S, Hefti A, Landmann R, Schifferli JA. Ectosomes released by human neutrophils are specialized functional units. *J Immunol* 1999;163:4564–4573.
20. Gasser O, Hess C, Miot S, Deon C, Sanchez JC, Schifferli JA. Characterisation and properties of ectosomes released by human polymorphonuclear neutrophils. *Exp Cell Res* 2003;285:243–257.
21. Sadallah S, Eken C, Schifferli JA. Ectosomes as modulators of inflammation and immunity. *Clin Exp Immunol* 2011;163:26–32.
22. Angelillo-Scherrer A. Leukocyte-derived microparticles in vascular homeostasis. *Circ Res* 2012;110:356–369.
23. Jara PI, Boric MP, Sáez JC. Leukocytes express connexin 43 after activation with lipopolysaccharide and appear to form gap junctions with endothelial cells after ischemia–reperfusion. *Proc. Natl. Acad. Sci. USA* 1995;92:7011–7015.
24. Eltzschig HK, Eckle T, Mager A, Küper N, Karcher C, Weissmüller T, Boengler K, Schulz R, Robson SC, Colgan SP. ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function. *Circ Res* 2006;99:1100–1108.
25. Branes MC, Contreras JE, Sáez JC. Activation of human polymorphonuclear cells induces formation of functional gap junctions and expression of connexins. *Med Sci Monit* 2002;8:BR313–BR323.
26. Zahler S, Hoffmann A, Gloe T, Pohl U. Gap–junctional coupling between neutrophils and endothelial cells: a novel modulator of transendothelial migration. *J Leukoc Biol* 2003;73:118–126.
27. Worthen GS, Schwab B III, Elson EL, Downey GP. Mechanics of stimulated neutrophils: cell stiffening induces retention in capillaries. *Science* 1989;245:183–186.
28. Ogura H, Tanaka H, Koh T, Fujita K, Fujimi S, Nakamori Y, Hosotsubo H, Kuwagata Y, Shimazu T, Sugimoto H. Enhanced production of endothelial microparticles with increased binding to leukocytes in patients with severe systemic inflammatory response syndrome. *J Trauma* 2004;56:823–830.
29. Lo SC, Hung CY, Lin DT, Peng HC, Huang TF. Involvement of platelet glycoprotein Ib in platelet microparticle mediated neutrophil activation. *J Biomed Sci* 2006;13:787–796.
30. Soriano AO, Jy W, Chirinos JA, Valdivia MA, Velasquez HS, Jimenez JJ, Horstman LL, Kett DH, Schein RM, Ahn YS. Levels of endothelial and platelet microparticles and their interactions with leukocytes negatively correlate with organ dysfunction and predict mortality in severe sepsis. *Crit Care Med* 2005;33:2540–2546.
31. Mostefai HA, Meziani F, Mastronardi ML, Agouni A, Heymes C, Sargentini C, Asfar P, Martinez MC, Andriantsitohaina R. Circulating

microparticles from patients with septic shock exert protective role in vascular function. *Am J Respir Crit Care Med* 2008;178:1148–1155.
32. Jy W, Mao WW, Horstman LL, Tao J, Ahn YS. Platelet microparticles bind, activate and aggregate neutrophils *in vitro*. *Blood Cells Mol Dis* 1995;21:217–231.
33. Forlow SB, McEver RP, Nollert MU. Leukocyte–leukocyte interactions mediated by platelet microparticles under flow. *Blood* 2000;95:1317–1323.
34. Muller I, Klocke A, Alex M, Kotzsch M, Luther T, Morgenstern E, Zieseniss S, Zahler S, Preissner K, Engelmann B. Intravascular tissue factor initiates coagulation via circulating microvesicles and platelets. *FASEB J* 2003;17:476–478.
35. Belizaire RM, Prakash PS, Richter JR, Robinson BR, Edwards MJ, Caldwell CC, Lentsch AB, Pritts TA. Microparticles from stored red blood cells activate neutrophils and cause lung injury after hemorrhage and resuscitation [abstract]. *J Am Coll Surg* 2012;214: 648–655.
36. Mesri M, Altieri DC. Endothelial cell activation by leukocyte microparticles. *J Immunol* 1998;161:4382–4387.
37. Mesri M, Altieri DC. Leukocyte microparticles stimulate endothelial cell cytokine release and tissue factor induction in a JNK1 signaling pathway. *J Biol Chem* 1999;274:23111–23118.
38. Dalli J, Norling LV, Renshaw D, Cooper D, Leung KY, Perretti M. Annexin 1 mediates the rapid anti-inflammatory effects of neutrophil-derived microparticles. *Blood* 2008;112:2512–2519.
39. Eken C, Sadallah S, Martin PJ, Treves S, Schifferli JA. Ectosomes of polymorphonuclear neutrophils activate multiple signaling pathways in macrophages. *Immunobiology* 2013;218:382–392.
40. Eken C, Martin PJ, Sadallah S, Treves S, Schaller M, Schifferli JA. Ectosomes released by polymorphonuclear neutrophils induce a MerTK-dependent anti-inflammatory pathway in macrophages. *J Biol Chem* 2010;285:39914–39921.
41. Pluskota E, Woody NM, Szpak D, Ballantyne CM, Soloviev DA, Simon DI, Plow EF. Expression, activation, and function of integrin αMβ2 (Mac-1) on neutrophil-derived microparticles. *Blood* 2008;112:2327–2335.
42. Watanabe J, Marathe GK, Neilsen PO, Weyrich AS, Harrison KA, Murphy RC, Zimmerman GA, McIntyre TM. Endotoxins stimulate neutrophil adhesion followed by synthesis and release of platelet-activating factor in microparticles. *J Biol Chem* 2003;278:33161–33168.
43. Mortaza S, Martinez MC, Baron-Menguy C, Burban M, de la Bourdonnaye M, Fizanne L, Pierrot M, Cales P, Henrion D, Andriantsitohaina R, *et al.* Detrimental hemodynamic and inflammatory effects of microparticles originating from septic rats. *Crit Care Med* 2009;37:2045–2050.
44. Guervilly C, Lacroix R, Forel JM, Roch A, Camoin-Jau L, Papazian L, Dignat-George F. High levels of circulating leukocyte microparticles are associated with better outcome in acute respiratory distress syndrome. *Crit Care* 2011;15:R31.
45. Kornerup KN, Salmon GP, Pitchford SC, Liu WL, Page CP. Circulating platelet–neutrophil complexes are important for subsequent neutrophil activation and migration. *J Appl Physiol* 2010;109:758–767.
46. Vaiyapuri S, Jones CI, Sasikumar P, Moraes LA, Munger SJ, Wright JR, Ali MS, Sage T, Kaiser WJ, Tucker KL, *et al.* Gap junctions and connexin hemichannels underpin hemostasis and thrombosis/clinical perspective. *Circulation* 2012;125:2479–2491.
47. Angelillo-Scherrer A, Fontana P, Burnier L, Roth I, Sugamele R, Brisset A, Morel S, Nolli S, Sutter E, Chassot A, *et al.* Connexin 37 limits thrombus propensity by downregulating platelet reactivity: clinical perspective. *Circulation* 2011;124:930–939.
48. Zarbock A, Singbartl K, Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet–neutrophil aggregation. *J Clin Invest* 2006;116:3211–3219.
49. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, Toy P, Werb Z, Looney MR. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* 2012;122:2661–2671.
50. van Rijen HVM, van Kempen MJA, Postma S, Jongsma HJ. Tumour necrosis factor α alters the expression of connexin 43, connexin 40, and connexin 37, in human umbilical vein endothelial cells. *Cytokine* 1998;10:258–264.
51. Ko YS, Yeh HI, Rothery S, Dupont E, Coppen SR, Severs NJ. Connexin make-up of endothelial gap junctions in the rat pulmonary artery as revealed by immunoconfocal microscopy and triple-label immunogold electron microscopy. *J Histochem Cytochem* 1999;47:683–691.
52. Wang L, Yin J, Nickles HT, Ranke H, Tabuchi A, Hoffmann J, Tabeling C, Barbosa-Sicard E, Chanson M, Kwak BR, *et al.* Hypoxic pulmonary vasoconstriction requires connexin 40–mediated endothelial signal conduction. *J Clin Invest* 2012;122:4218–4230.
53. Chanson M, Derouette JP, Roth I, Foglia B, Scerri I, Dudez T, Kwak BR. Gap junctional communication in tissue inflammation and repair. *Biochim Biophys Acta* 2005;1711:197–207.
54. Sarieddine MZ, Scheckenbach KEL, Foglia B, Maass K, Garcia IN, Kwak BR, Chanson M. Connexin 43 modulates neutrophil recruitment to the lung. *J Cell Mol Med* 2009;13:4560–4570.
55. Véliz LP, González FG, Duling BR, Sáez JC, Boric MP. Functional role of gap junctions in cytokine-induced leukocyte adhesion to endothelium *in vivo*. *Am J Physiol Heart Circ Physiol* 2008;295:H1056–H1066.
56. Nolan S, Dixon R, Norman K, Hellewell P, Ridger V. Nitric oxide regulates neutrophil migration through microparticle formation. *Am J Pathol* 2008;172:265–273.
57. Hidalgo A, Chang J, Jang JE, Peired AJ, Chiang EY, Frenette PS. Heterotypic interactions enabled by polarized neutrophil microdomains mediate thromboinflammatory injury. *Nat Med* 2009;15:384–391.
58. Hyun YM, Sumagin R, Sarangi PP, Lomakina E, Overstreet MG, Baker CM, Fowell DJ, Waugh RE, Sarelius IH, Kim M. Uropod elongation is a common final step in leukocyte extravasation through inflamed vessels. *J Exp Med* 2012;209:1349–1362.
59. Walker DC, Behzad AR, Chu F. Neutrophil migration through preexisting holes in the basal laminae of alveolar capillaries and epithelium during streptococcal pneumonia. *Microvasc Res* 1995;50: 397–416.
60. Zhang P, Su J, Mende U. Cross-talk between cardiac myocytes and fibroblasts: from multi-scale investigative approaches to mechanisms and functional consequences. *Am J Physiol Heart Circ Physiol* 2012; 303:H1385–H1396.
61. Bastarache JA, Fremont RD, Kropski JA, Bossert FR, Ware LB. Procoagulant alveolar microparticles in the lungs of patients with acute respiratory distress syndrome. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L1035–L1041.
62. Mutschler DK, Larsson AO, Basu S, Nordgren A, Eriksson MB. Effects of mechanical ventilation on platelet microparticles in bronchoalveolar lavage fluid. *Thromb Res* 2002;108:215–220.
63. Chanson M, Berclaz PY, Scerri I, Dudez T, Wernke-Dollries K, Pizurki L, Pavirani A, Fiedler MA, Suter S. Regulation of gap junctional communication by a pro-inflammatory cytokine in cystic fibrosis transmembrane conductance regulator–expressing but not cystic fibrosis airway cells. *Am J Pathol* 2001;158:1775–1784.
64. Scerri I, Tabary O, Dudez T, Jacquot J, Foglia B, Suter S, Chanson M. Gap junctional communication does not contribute to the interaction between neutrophils and airway epithelial cells. *Cell Commun Adhes* 2006;13:1–12.