Endocytosis and intracellular processing of platelet microparticles by brain endothelial cells

Dorothée Faille a, b, *, Fatima El-Assaad a, Andrew J. Mitchell a, c, Marie-Christine Alessi d, Giovanna Chimini c, Thierry Fusai b, Georges E. Grau a, #, Valéry Combes a, #

a Department of Pathology, University of Sydney, Camperdown, Australia
b Unité de Recherche en Biologie et Epidémiologie Parasitaires, UMR6236-IFR48, Antenne Marseille de l’Institut de Recherche Biomédicale des Armées, Marseille, France
c CIML, Inserm U631, CNRS UMR6102, Aix-Marseille Université, Faculté des Sciences, Marseille, France
d Inserm U626, Aix-Marseille Université, Faculté de Médecine, Marseille, France

Received: May 23, 2011; Accepted: August 23, 2011

Abstract

Platelet-derived microparticles (PMP) bind and modify the phenotype of many cell types including endothelial cells. Recently, we showed that PMP were internalized by human brain endothelial cells (HBEC). Here we intend to better characterize the internalization mechanisms of PMP and their intracellular fate. Confocal microscopy analysis of PKH67-labelled PMP distribution in HBEC showed PMP in early endosome antigen 1 positive endosomes and in LysoTracker-labelled lysosomes, confirming a role for endocytosis in PMP internalization. No fusion of calcein-loaded PMP with HBEC membranes was observed. Quantification of PMP endocytosis using flow cytometry revealed that it was partially inhibited by trypsin digestion of PMP surface proteins and by extracellular Ca²⁺/H11001 chelation by EDTA, suggesting a partial role for receptor-mediated endocytosis in PMP uptake. This endocytosis was independent of endothelial receptors such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 and was not increased by tumour necrosis factor stimulation of HBEC. Platelet-derived microparticle internalization was dramatically increased in the presence of decomplemented serum, suggesting a role for PMP opsonin-dependent phagocytosis. Platelet-derived microparticle uptake was greatly diminished by treatment of HBEC with cytochalasin D, an inhibitor of microfilament formation required for both phagocytosis and macropinocytosis, with methyl-β-cyclodextrin that depletes membrane cholesterol needed for macropinocytosis and with amiloride that inhibits the Na⁺/H⁺ exchanger involved in macropinocytosis. In conclusion, PMP are taken up by active endocytosis in HBEC, involving mechanisms consistent with both phagocytosis and macropinocytosis. These findings identify new processes by which PMP could modify endothelial cell phenotype and functions.

Keywords: endothelial cells • inflammation • internalization • macropinocytosis • microparticles • phagocytosis

Introduction

Microparticles (MP) are submicron membrane fragments released from many cell types upon activation or apoptosis. Their composition and functional properties vary with their cellular origin and the type of stimulus involved in their formation. Circulating MP mainly derive from platelets. Elevated levels of PMP have been associated with numerous pathological states such as atherothrombosis [1] or infectious diseases [2, 3]. Platelet-derived microparticle interactions with various cell types and their functional consequences have been described in many studies. Indeed, PMP modulate biological functions of endothelial cells, leucocytes or haemopoietic cells via antigens, bioactive lipids or chemokines that they transport [4–11].

Less attention has been paid to the fate of PMP and neither their life span nor the mechanisms of their clearance have been clearly determined yet. Several groups suggested that PMP were cleared from the circulation very quickly following infusion into animal [12, 13] and, more recently, lactadherin has been identified as one of the mediators of the clearance of phosphatidylserine (PS)-expressing PMP by splenic macrophages [14].

© 2011 The Authors
Journal of Cellular and Molecular Medicine © 2011 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

doi: 10.1111/j.1582-4934.2011.01434.x
In a recent study, we showed that PMP were internalized by HBEC by an active process and induced changes in the surface phenotype of these cells that could have a pivotal role in the pathogenesis of cerebral malaria, an acute encephalopathy secondary to the infection by Plasmodium falciparum [15]. However, this internalization process had not been characterized. Here, using fluorescent markers for several endocytic compartments and various specific endocytosis inhibitors, we further characterize the intracellular sorting of PMP in HBEC and the mechanisms involved in this endocytosis. These analyses may help not only to elucidate the mechanisms by which PMP are taken up and degraded or recycled by cells, but also provide new insights in the regulation of the transport of biomolecules by PMP into target cells.

**Materials and methods**

**Microvascular endothelial cells**

Human brain endothelial cells, HBEC-5i, were derived by Dorovini-Zis *et al.* from small fragments of human cerebral cortex obtained from patients who had died of various causes [16]. These immortalized cells were then characterized and maintained in our laboratory, as described elsewhere [15, 17].

**Platelet microparticles**

Platelet-derived microparticles were purified from *in vitro*-activated platelet supernatants and labelled with red PKH26 or green PKH67 (Sigma-Aldrich, St. Louis, MO, USA) as previously described [15]. Briefly, washed platelets from human blood were incubated for 45 min. at 37°C in the presence of calcium ionophore A23187 (10 μM) and platelets and cell debris were removed by serial centrifugations (6 min. at 2000 × g and 2 min. at 13,000 × g). PMP were pelleted by centrifugation at 60 min. at 20,800 × g and resuspended in PBS. Platelet-derived microparticles were then mixed 1:1 with volume to volume with PKH by gentle pipetting for 1 min. and washed with DMEM containing 10% fetal calf serum (FCS). Then, PMP were labelled with red PKH26 or green PKH67 (Sigma-Aldrich, St. Louis, MO, USA). Alternatively, PMP were directly incubated with 10 μg/ml calcine AM (Invitrogen, San Jose, CA, USA) for 60 min. at 37°C and washed twice in PBS. Labelled PMP were counted by flow cytometry. Supernatants resulting from the final wash were used as a control.

In some experiments, to remove protease-sensitive glycoproteins from the surface of PMP, we performed digestion by 0.25% (w/v) trypsin (Gibco) for 30 min. at 37°C as described by Baj-Krzyworzeka *et al.* [18].

**Identification of the endocytic compartments containing PMP**

Human brain endothelial cells grown to confluence on gelatin-coated 8-well labtek slides (Nunc) were incubated for 90 min. total incubation time at 37°C with PKH67-labelled PMP and washed to remove unbound PMP. LysoTracker Red DND-99 (50 nM; Invitrogen) or ER-Tracker Red (100 nM; Invitrogen), for the labelling of lysosomes or endoplasmic reticulum respectively, were added 30 min. before washing and fixation. For early endosome labelling, HBEC were fixed with 4% (v/v) paraformaldehyde for 10 min. and permeabilized with Triton X-100 0.1% (v/v) in PBS 5 min. at room temperature. Non-specific binding was blocked by incubation for 30 min. in PBS containing 1% (w/v) BSA. Early endosome antigen 1 (EEA-1) was detected using a mouse monoclonal IgG1 to EEA-1 (clone 14, 1:1000; BD Transduction Laboratories, San Jose, CA, USA) revealed with Alexa-Fluor 555 conjugated goat anti-mouse secondary antibody (1:400; Invitrogen). Both primary and secondary antibodies were diluted in 1% (w/v) BSA in PBS and incubated for 1 hr at room temperature. Finally, cells were washed and kept in PBS for analysis. Samples were analysed by confocal laser scanning microscopy using an Axiovert 200 inverted microscope, equipped with a Zeiss LSM 510 scanning module and Zeiss LSM510 Software (Zeiss, Oberkochen, Germany).

**PMP endocytosis by HBEC and inhibition assays**

To quantify PMP endocytosis by HBEC, cells were grown to confluence in 96-well plates and were either left unstimulated or incubated overnight with 10 ng/ml tumour necrosis factor (TNF). Cell monolayers were then washed in serum-free medium and further incubated for 90 min. at 37°C with PKH67-labelled PMP (2000/μl) in serum-free DMEM.

To evaluate the effect of serum in PMP endocytosis, medium was supplemented with 10% foetal calf serum (FCS; Gibco) depleted by heating for 30 min. at 56°C.

In some experiments, the calcium-chelator EDTA was added to the medium to a final concentration of 2.5 mM during endocytosis assay to investigate the role of extracellular calcium in PMP endocytosis.

To evaluate the role of endothelial surface adhesion receptors in PMP internalization, HBEC were first pre-incubated for 30 min. at 4°C with mAbs against vascular cell adhesion molecule-1 (VCAM-1, CD106, clone 1G11, IgG1) and intercellular adhesion molecule-1 (ICAM-1, CD54, clone 84H10, IgG1) purchased from Beckman Coulter Immunotech. An irrelevant isotype-matched mAb (clone 679.1Mc7) was used as control.

To evaluate the effects of specific endocytosis inhibitors, HBEC were first pre-incubated with the following inhibitors in serum-free medium for 30 min. at 37°C: 10 μM cytochalasin D, 10 mM methyl-β-cyclodextrin from Sigma-Aldrich, calcium ionophore A23187 (10 μM), either pre-incubated with the following inhibitors in serum-free medium for 30 min. at 37°C: 10 μM cytochalasin D, 10 mM methyl-β-cyclodextrin and 250 μM amiloride. Cytochalasin D and amiloride were kept in the medium during the endocytosis assay. To avoid any effect of cholesterol depletion on PMP integrity, a washing step to remove methyl-β-cyclodextrin from the prior co-incubation medium was necessary before the addition of PMP and the endocytosis assay was carried out in the absence of this inhibitor. Cells were incubated with 0.5 mg/ml 7-KD-derivatized albumin (Sigma-Aldrich) at 37°C for 30 min. as a positive control for macropinocytosis.

Inhibitor and EDTA were tested at different concentrations to establish working concentrations for maximum inhibition without toxic effects on cells. After incubation, HBEC were washed, detached by a short trypsin treatment and analysed for fluorescence by flow cytometry. To assess effective activation of HBEC by TNF, detached HBEC were analysed for surface up-regulation of VCAM-1 and ICAM-1 by flow cytometry as previously described [17]. For all these experimental conditions, flow cytometry analyses were performed with live cells using an Epics XL flow cytometer (Beckman Coulter Immunotech). Data were obtained and analysed using System2 software (Beckman Coulter Immunotech). Live cells were gated by forward/side scattering from a total of 10,000 events.

**Statistical analyses**

Continuous variables were tested for normal distribution with the Kolmogorov–Smirnov test and are presented as continuous variables as
mean ± S.D. Means between two groups were compared using a two-tailed unpaired Student’s *t*-test. *P* values less than 0.05 were accepted as significant. All statistical analyses were performed with GraphPad Prism version 4.0 for Windows Software.

**Results**

**Subcellular localization of internalized PMP**

We previously demonstrated that PMP were internalized by HBEC in a time-dependent and temperature-sensitive process [15]. Once internalized, PMP were found to be surrounded by membranes labelled with Alexa-555 coupled wheat germ agglutinin, suggesting that PMP moved into large intracellular vesicles but did not stay along the plasma membrane.

To evaluate the potential role of endocytosis in PMP uptake, we first identified the intracellular vesicles in which PMP were processed after internalization. Using confocal microscopy, the cell distribution of PHK67-labelled PMP was compared to that of endosomal structures, as revealed by EEA1 and LysoTracker red staining. As shown in Figure 1, after 90 min. of co-incubation, 15% of PMP were found in EEA1-positive vesicles (top) and most PMP co-localized with LysoTracker red fluorescence in HBEC (middle). These data clearly indicate that PMP are internalized by an endocytic process and that, after uptake, PMP traffic along the endosomal compartment through early endosomes towards more acidic lysosomes. Endocytosed PMP showed no colocalization...
with ER-Tracker staining (bottom), indicating that endocytosed PMP did not enter the retrograde-transport pathway to the endoplasmic reticulum at that time.

Detection of PMP fusion with HBEC membrane by calcein release

To search for PMP fusion with the endothelial membrane and to detect a possible PMP fragmentation once internalized, PMP content was stained with soluble calcein AM (green) in addition to the membranous PKH26 labelling (red). After staining, 48% of PMP were double labelled for both calcein and PKH26 (Fig. 2A). Figure 2B shows that, after PMP internalization by HBEC, no PKH26 was transferred from PMP to endothelial membrane and no entrapped calcein was released within the cytosol, suggesting that PMP content did not diffuse inside the target cell but remained co-localized with PMP membranes. This observation clearly indicates that PMP internalization by HBEC does not involve the fusion of PMP lipids with the endothelial plasma membrane but, rather, an uptake of PMP material with subsequent localization in intracellular vesicles.

Mechanisms of PMP endocytosis

To further characterize PMP internalization by HBEC, we tested the effect of different modulators added just before or during the internalization assay and quantified the uptake of PKH67-labelled PMP by flow cytometry (Fig. 3A). Firstly, PMP internalization was only reduced by 38% after trypsin digestion (Fig. 3B), indicating that, in addition to the trypsin-sensitive protein components of PMP, other trypsin-resistant components such as lipids can be responsible for their interaction with HBEC. Secondly, pre-treatment of HBEC with EDTA resulted in a significant 33% decrease of PMP uptake (Fig. 3B), suggesting that this phenomenon is partially calcium dependent. Thirdly, we showed that PMP internalization was not affected when HBEC were first incubated with mAbs against VCAM-1 or ICAM-1. Moreover, PMP internalization was not increased in TNF-pre-stimulated HBEC as compared to resting cells (Fig. 3B). This suggests that PMP internalization is not mediated by TNF-up-regulated adhesion receptors such as VCAM-1 or ICAM-1. Surprisingly, in the presence of decomplemented FCS, there was a threefold increase in PMP uptake (Fig. 3B), suggesting a role for PMP opsonization by decomplemented FCS in their entry into HBEC.

To determine which of the several possible cellular uptake mechanisms were responsible for PMP internalization, the effects of three different endocytosis inhibitors were examined. These inhibitors were also tested on the entry of 70-kD FITC-dextran, a marker of macropinocytosis, for comparison. Cytochalasin D was used to inhibit actin elongation and the formation of microfilaments and microtubules, methyl-β-cyclodextrin to deplete membrane cholesterol and inhibit lipid raft-dependent pathways, and amiloride was tested to inhibit the Na+/H+ exchanger required for macropinocytosis [19]. In HBEC, 70 kD FITC-dextran endocytosis was reduced by methyl-β-cyclodextrin and amiloride, as expected for macropinocytosis (Fig. 3C, grey bars). Cytochalasin D was found to produce an unexpected increase in dextran entry into HBEC, indicating an alteration in macropinocytosis with this inhibitor. In contrast, all drugs tested were found to partially inhibit the entry of PMP into HBEC (Fig. 3C, black bars).

Discussion

A growing number of studies report the cellular uptake of MP by mononuclear haematopoietic cells, monocytes, neutrophils, plasmacytoid dendritic cells [18, 20–23] and by endothelial cells [15, 24–26]. However, the mechanisms involved in this process have not been clearly defined yet.
In this study, we have examined the intracellular localization of PMP internalized by brain microvascular endothelial cells and attempted to decipher the pathways involved in this phenomenon. Using markers for endosomal compartments, we demonstrated for the first time that internalized PMP were distributed within endosomes and lysosomes after cell entry, supporting the fact that endocytosis was the predominant route of PMP entry into endothelial cells. This major finding differs from the recent work by Terrisse et al. who did not find any co-localization of endocytosed endothelial-derived MP with markers of early or late endosomes after 2 hrs of co-incubation with HUVEC [25], suggesting that either kinetics or pathways for MP intracellular processing might vary between different endothelial cell types.

Although PMP were efficiently endocytosed by endothelial cells, they did not fuse with their plasma membrane because no diffusion of calcein-labelled PMP content occurred inside the target cell cytosol. This is consistent with the observation of Aharon et al. who found that monocyte-derived MP bound to and were ingested by HUVEC without any fusion of MP with the cell membrane [24] but contrasts with the fusion of monocyte-derived MP with activated platelets described by del Conde et al. [27]. These results suggest that MP interaction might involve different mechanisms depending on the target cell type.

Endocytosis may occur through several distinct mechanisms, usually divided into two broad categories: phagocytosis, a process restricted to specialized mammalian cells, and pinocytosis, which occurs in all mammalian cells and encompasses macropinocytosis, clathrin-mediated endocytosis, and caveolae-mediated endocytosis [28]. Although clathrin- and caveolin-dependent endocytosis involve the uptake of particles smaller than 100 nm in diameter, phagocytosis and macropinocytosis involve the uptake of larger volumes. Because MP are defined as particles with a diameter varying between 100 and 1000 nm [6], we focused on the potential role for phagocytosis or macropinocytosis in PMP uptake.

Although phagocytosis requires cell-surface recognition of particles and proceeds via sequential engagement of receptors, macropinocytosis occurs via non-selective membrane uptake. We thus tried to determine which type of MP surface components and endothelial receptors could be involved in MP endocytosis by HBEC. The partial inhibition of PMP internalization after treatment of PMP with trypsin indicates that protease-sensitive components of PMP, such as surface glycoproteins, play a minor role in PMP binding and further internalization but rather supports a role for other components such as lipids. PMP uptake was slightly inhibited by EDTA, suggesting that Ca^{2+}-dependent (usually
extensive membrane reorganization and are reduced by cytochalasin D treatment [28]. Thus, the reduction of PMP entry into endothelial cells by cytochalasin D does not allow us to identify one of the two processes as a major pathway for PMP entry into HBEC. The decrease of PMP uptake by the cholesterol depleting agent methyl-β-cyclodextrin shows that PMP endocytosis depends on cholesterol content and suggests a role for macropinocytosis because cholesterol is required for membrane ruffling and macropinocytosis [41]. It should be stressed out that, in addition to cholesterol depletion, methyl-β-cyclodextrin has pleiotropic effects on different membrane components, such as phospholipids, that could have a potential role in PMP endocytosis. The incomplete inhibition of PMP entry by the Na+/K+ transporter inhibitor amiloride indicates that endocytic uptake of PMP occurs partially by non-selective macropinocytosis [19]. The unique susceptibility of macropinocytosis to inhibitors of Na+/H+ exchange has been extensively used to differentiate it from other types of endocytosis. However, caution should be made in interpreting these data because the inhibition of macropinocytosis by amiloride could also result from its effects on cell pH [42].

Phagocytosis of particles, apoptotic bodies and aged erythrocytes is predominately attributed to macrophages, neutrophils and dendritic cells. However, several studies have described the uptake of large objects by endothelial cells, including apoptotic bodies [43–46] and MP [24–26]. More surprising is the endocytic activity of endothelial cells derived from the brain. Indeed, the most important function of endothelial cells that line the brain capillaries is to form the blood–brain barrier (BBB), which physically separates blood components from the brain and the cerebrospinal fluid. This barrier is based, in part, on a paucity of vesicular activity within the brain microvascular endothelial cells that limits endocytosis. However, endocytosis can be modulated by vasoactive substances, such as angiotensin and bradykinin [47], and cytokines, such as TNF [48], and may dramatically increase in disease states, rendering the BBB less restrictive for blood-borne proteins and cells.

Understanding PMP endocytotic process could provide essential clues that may reveal how endothelial cells can differentially regulate the transport of different components across the BBB. This is likely to shed light on the processes underlying numerous neurological disorders during which an increased production of PMP at the interface of the BBB occurs. For example, PMP carry agents implicated in neurodegenerative disorders such as amyloid β-protein precursor present in amyloid plaques in Alzheimer's disease [49] and PMP could actively transport these agents across the BBB [50].

Overall, our findings provide new mechanisms by which MP interact with endothelial cells and could modify their phenotype and functions. This could help understand the role of MP in the broad range of physiological and pathological processes during which MP levels are increased. MP uptake by endothelial cells could also participate in MP clearance from the circulation.
Acknowledgements

The authors are indebted to the Plate-forme d’Imagerie Commune Scientifique de Luminy for expert technical assistance. This work was supported by grants from the National Health and Medical Research Council of Australia (NHMRC Project Grant No. 464983; www.nhmrc.gov.au), the Australian Research Council (ARC Discovery Project No. DP0774425; www.arc.gov.au) and from the European Union-Health and Medical Research Council of Australia (EU-NHMRC Project Grant No. 512101; europa.eu), the Rebecca L. Cooper Foundation (www.cooperfoundation.org.au), the University of Sydney Major Equipment Grant scheme (www.usyd.edu.au) and the AL Kerr Bequest, Sydney Medical School. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors’ contributions

DF performed the research. DF, FEA, AM, GEG and VC designed the research, analysed the data and wrote the paper. MCA, GC and TF provided material and analytical tools.

Conflict of interest

The authors confirm that there are no conflicts of interest.

References

1. Tan KT, Lip GY. The potential role of platelet microparticles in atherosclerosis. Thromb Haemost. 2005; 94: 488–92.
2. Nieuwland R, Berkmanns RJ, McGregor S, et al. Cellular origin and procoagulant properties of microparticles in meningo-coccal sepsis. Blood. 2000; 95: 930–5.
3. Pankoui Mfonkeu JB, Gouado I, Fotso Kuate H, et al. Elevated cell-specific microparticles are a biological marker for cerebral dysfunctions in human severe malaria. PLoS One. 2010; 5: e13415.
4. Barry OP, Pratico D, Lawson JA, et al. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. J Clin Invest. 1997; 99: 2118–27.
5. Barry OP, Pratico D, Savani RC, et al. Modulation of monocyte-endothelial cell interactions by platelet microparticles. J Clin Invest. 1998; 102: 136–44.
6. Jay W, Mao WW, Horstman L, et al. Platelet microparticles bind, activate and aggregate neutrophils in vitro. Blood Cells Mol Dis. 1995; 21: 217–31; discussion 231a.
7. Forlow SB, McEver RP, Nollert MU. Leukocyte-leukocyte interactions mediated by platelet microparticles under flow. Blood. 2000; 95: 1317–23.
8. Salanova B, Choi M, Rolle S, et al. Beta2-integrins and acquired glycoprotein lib/llla (GPlib/llla) receptors cooperate in NF-kappaB activation of human neutrophils. J Biol Chem. 2007; 282: 27960–9.
9. Janowska-Wieczorek A, Majka M, Kijowski J, et al. Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment. Blood. 2001; 98: 3143–9.
10. Mause SF, von Hundelshausen P, Zernecke A, et al. Platelet microparticles: a transcellular delivery system for RANTES promoting monocyte recruitment on endothelium. Arterioscler Thromb Vasc Biol. 2005; 25: 1512–8.
11. Mause SF, Ritzel E, Liehn EA, et al. Platelet microparticles enhance the vasoregenerative potential of angiogenic early outgrowth cells after vascular injury. Circulation. 2010; 122: 495–506.
12. Rand ML, Wang H, Bang KW, et al. Rapid clearance of procoagulant platelet-derived microparticles from the circulation of rabbits. J Thromb Haemost. 2006; 4: 1621–3.
13. Flaumenhaft R. Formation and fate of platelet microparticles. Blood Cells Mol Dis. 2006; 36: 182–7.
14. Dasgupta SK, Abdel-Monem H, Niravath P, et al. Lactadherin and clearance of platelet-derived microvesicles. Blood. 2009; 113: 1332–9.
15. Faille D, Combes V, Mitchell AJ, et al. Platelet microparticles: a new player in endothelial cell interaction under flow. J Thromb Haemost. 2010; 8: 2810–9.
16. Dorovini-Zis K, Prameya R, Bowman PD. Culture and characterization of microparticles in meningo-coccal sepsis. Thromb Haemost. 2008; 99: 86–95.
17. Gasser O, Schifferli JA. Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. Blood. 2004; 104: 2543–8.
18. Angelot F, Seilles E, Bichie S, et al. Endothelial cell-derived microparticles induce plasmacytoid dendritic cell maturation: potential implications in inflammatory diseases. Haematologica. 2009; 94: 1502–12.
19. Aharon A, Tamari T, Brenner B. Monocyte-derived microparticles and exosomes induce procoagulant and apoptotic effects on endothelial cells. Thromb Haemost. 2008; 100: 678–85.
20. Terrisse AD, Puech N, Allart S, et al. Internalization of microparticles by endothelial cells promotes platelet/endothelial cell interaction under flow. J Thromb Haemost. 2010; 8: 2810–9.
21. Rautou PE, Leroyer AS, Ramkhalawon B, et al. Microparticles from human atherosclerotic plaques promote endothelial ICAM-1-dependent monocyte adhesion...
and transendothelial migration. Circ Res. 2011; 108: 335–43.
27. Del Conde I, Shrimpton CN, Thiagarajan P, Lopez JA. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. Blood. 2005; 106: 1604–11.
28. Conner SD, Schmid SL. Regulated portals of entry into the cell. Nature. 2003; 422: 37–44.
29. Grau GE, Tacchini-Cottier F, Vesin C, et al. TNF-induced microvascular pathology: active role for platelets and importance of the LFA-1/ICAM-1 interaction. Eur Cytokine Netw. 1993; 4: 415–9.
30. Lou J, Donati YR, Juillard P, et al. Platelets play an important role in TNF-induced microvascular endothelial cell pathology. Am J Pathol. 1997; 151: 1397–405.
31. Mannel DN, Grau GE. Role of platelet adhesion in homeostasis and immunopathology. Mol Pathol. 1997; 50: 175–85.
32. Pereira J, Palomo I, Ocqueteau M, et al. Platelet aging in vivo is associated with loss of membrane phospholipid asymmetry. Thromb Haemost. 1999; 82: 1318–21.
33. Maugeri N, Rovere-Querini P, Evangelista V, et al. Neutrophils phagocytose activated platelets in vivo: a phosphatidylycerine, P-selectin, and β2 integrin-dependent cell clearance program. Blood. 2009; 113: 5254–65.
34. Brown SB, Clarke MC, Magowan L, et al. Constitutive death of platelets leading to scavenger receptor-mediated phagocytosis. A caspase-independent cell clearance program. J Biol Chem. 2000; 275: 5987–96.
35. Abdel-Monem H, Dasgupta SK, Le A, et al. Phagocytosis of platelet microvesicles and beta2-glycoprotein I. Thromb Haemost. 2010; 104: 335–41.
36. Willekens FL, Werre JM, Krujt JK, et al. Liver Kupffer cells rapidly remove red blood cell-derived vesicles from the circulation by scavenger receptors. Blood. 2005; 105: 2141–5.
37. Goli D, Hrenjak A, Levak-Frank S, et al. Scavenger receptor class B, type I is expressed in porcine brain capillary endothelial cells and contributes to selective uptake of HDL-associated vitamin E. J Neurochem. 2001; 76: 498–508.
38. Husemann J, Loike JD, Anankov R, et al. Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. Glia. 2002; 40: 195–205.
39. Setty BN, Betal SG. Microvascular endothelial cells express a phosphatidylyserine receptor: a functionally active receptor for phosphatidylycerine-positive erythrocytes. Blood. 2008; 111: 905–14.
40. Litvack ML, Post M, Palaniyar N. IgM promotes the clearance of small particles and apoptotic microparticles by macrophages. PLoS One. 2011; 6: e17223.
41. Grimmer S, van Deurs B, Sandvig K. Membrane ruffling and macropinocytosis in A431 cells require cholesterol. J Cell Sci. 2002; 115: 2953–62.
42. Koivusalo M, Welch C, Hayashi H, et al. Amiloride inhibits macropinocytosis by lowering submembranous pH and preventing Ral and Cdc42 signaling. J Cell Biol. 2010; 188: 547–63.
43. Fens MH, Mastrobattista E, de Graaff AM, et al. Angiogenic endothelium shows lactadherin-dependent phagocytosis of aged erythrocytes and apoptotic cells. Blood. 2008; 111: 4542–50.
44. Hess KL, Tudor KS, Johnson JD, et al. Human and murine high endothelial venule cells phagocytose apoptotic leukocytes. Exp Cell Res. 1997; 236: 404–11.
45. Dini L, Pagliara P, Carla EC. Phagocytosis of apoptotic cells by liver: a morphological study. Microsc Res Tech. 2002; 57: 530–40.
46. Kirsch T, Woywodt A, Beese M, et al. Engagement of apoptotic cells by microvascular endothelial cells induces proinflammatory responses. Blood. 2007; 109: 2854–62.
47. Guillot FL, Audus KL. Angiotensin peptide regulation of fluid-phase endocytosis in brain microvesSEL endothelial cell monolayers. J Cereb Blood Flow Metab. 1990; 10: 827–34.
48. Defazio G, Trojano M, Ribatti D, et al. ICAM 1 expression and fluid phase endocytosis of cultured brain microvascular endothelial cells following exposure to interferon beta-1a and TNFalpha. J Neuroimmunol. 1998; 88: 13–20.
49. Nomura S, Komiyama Y, Miyake T, et al. Amyloid beta-protein precursor-rich platelet microparticles in thrombotic disease. Thromb Haemost. 1994; 72: 519–22.
50. Horstman LL, Jy W, Minagar A, et al. Cell-derived microparticles and exosomes in neuroinflammatory disorders. Int Rev Neurobiol. 2007; 79: 227–68.