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CELL-DERIVED MICROPARTICLES:

A NEW CHALLENGE IN NEUROSCIENCE

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Abbreviations used: ABCA1, ATP-binding cassette transporter A1; APC, activated protein C; EGFR, epidermal growth factor receptor; EPCR, endothelial protein C receptor; ITP, idiopathic thrombocytopenic purpura; MPs, microparticles; MS, multiple sclerosis; PS, phosphatidylserine; TF, tissue factor; TIA, transient ischemic attack; TNF-α, tumor necrosis factor α; uPA, urokinase-type plasminogen activator.

Abstract

Microparticles (MPs) are membrane fragments shed by cells activated by a variety of stimuli including serine proteases, inflammatory cytokines, growth factors and stress inducers. MPs originating from platelets, leukocytes, endothelial cells and erythrocytes are found in circulating blood at relative concentrations determined by the pathophysiological context. The procoagulant activity of MPs is their most characterized property as a determinant of thrombosis in various vascular and systemic diseases including myocardial infarction and diabetes. An increase in circulating MPs has also been associated with ischemic cerebrovascular accidents, transient ischemic attacks, multiple sclerosis and cerebral malaria. Recent data indicate that besides their procoagulant components and identity antigens, MPs bear a number of bioactive effectors that can be disseminated, exchanged and transferred via MPs-cell interactions. Furthermore, since activated parenchymal cells may also shed MPs carrying identity antigens and biomolecules, MPs are now emerging as new messengers/biomarkers from a specific tissue undergoing activation or damage. Thus, detection of MPs of neurovascular origin in biological fluids such as CSF or tears, and even in circulating blood in case of blood brain barrier leakage, would not only improve our comprehension of neurovascular pathophysiology, but may also constitute a powerful tool as a biomarker in disease prediction, diagnosis, prognosis and follow-up.

Running title: Microparticles in neuroscience

Key words: microparticles, hemostasis, neurovascular unit, ischemic stroke, multiple sclerosis, cerebral malaria.

Introduction

Brain functions and survival of its multiple cellular components depend on blood flowing through a patent vascular microcirculation that ensures selective exchange of vital elements (e.g. glucose, oxygen and hormones), excretion of metabolic products and transmigration of competent cells. The structural/functional-integrated network that regulates these physiological functions is the neurovascular unit.

It is now well known that a number of stress conditions and inflammatory mediators may stimulate and activate vascular and blood cells. One of the earliest manifestations of cell activation is plasma membrane blebbing and shedding into body fluids of membrane fragments known and designated hereafter as microparticles (MPs) (Freyssinet 2003) –other designations in the literature are: microvesicles, ectosomes, shedding vesicles, exovesicles. Plasma membrane remodelling is an early event observed in cells entering apoptosis as well. MPs characteristically display procoagulant properties and behave also as a storage pool of bioactive molecular effectors, messengers of cell activation and apoptosis (Morel *et al.* 2004c).

Cells in the neurovascular unit or its vicinity including the endothelial lining and neural cells (neurons, astrocytes, oligodendrocytes and microglia) are also subjected to stress by a variety of stimuli (e.g. oxygen radicals, inflammation, ischemia…) known to induce membrane shedding in vascular cells. It is therefore possible that shedding of cellular MPs in the neurovascular network may be linked to the onset and progression of a variety of CNS diseases including stroke, vascular dementia, inflammatory and age-related neurodegenerative disorders. In this review we analyze the state of the art on MPs in the CNS and provide clues that may improve our knowledge in the field. The review also examines MP detection and

characterization as possible tools for identification of new markers and biological signal conveyors in stroke and other CNS diseases.

Circulating blood microparticles: haemostatic and inflammatory effectors

Plasma membrane blebbling and shedding of microparticles

The most characterized cellular MPs are those originating from platelets, leukocytes, erythrocytes and endothelial cells, detected in circulating blood (Morel *et al.* 2006). A number of studies have demonstrated that stimulation of these cells triggers a characteristic activation pattern of events: increased levels of cytoplasmic calcium associated to translocation of phosphatidylserine (PS) from the inner to the outer leaflet of the membrane and activation of calpains that, by cleaving cytoskeletal filaments, facilitate MP shedding (Pasquet *et al.* 1996). The increase in intracellular calcium concentration induces a disordered state in the phospholipid membrane asymmetry of quiescent cells that is maintained by the concerted activity of lipid transporter proteins (Bevers *et al.* 1999, Daleke 2003). The ATP-dependent flippases (e.g. aminophospholipid translocase) and floppases (including the ATP-binding cassette transporter A1, ABCA1) are respectively inward- and outward-directed transporters, whereas the calcium-dependent scramblases (e.g. phospholipid scramblase receptor-1, PLSCR-1) facilitate bidirectional movement between the membrane leaflets (Fig. 1). The rate of PS translocation has been found to be sensitive to the altered expression of ABCA1 in knock-out mice (Hamon *et al.* 2000).

In summary, the mechanism governing the plasma membrane PS redistribution is of a complex nature and implicates various membrane (lipid transporters, receptors, calcium channels) and cytoplasmic (cytoskeleton, calpains) actors (Zwaal *et al.* 2005). Recent data indicate that the formation and integrity of lateral transient membrane microdomains termed

rafts, rich in cholesterol and sphingolipids (Hancock 2006, Brown 2006), may provide an appropriate platform for the assembly of some of these regulatory elements and be essential for the transmembrane redistribution of PS (Sun *et al.* 2002, Kunzelmann-Marche *et al.* 2002, Lopez *et al.* 2005). PS exposure was indeed colocalized with membrane lipid raft regions (Fischer *et al.* 2006). Rafts are dynamic features that may appear and disappear as a function of the state of activation and types of stimuli (Hancock 2006). Raft microdomains in the outer leaflet of the plasma membrane are coupled to microdomains in the inner leaflet that contain signalling kinases, able to initiate transmembrane signalling transduction pathways (Michel & Bakovic 2007).

Consequences of phosphatidylserine exposure

Transfer of PS to the outer leaflet of the membrane is an early sign of cell activation or apoptosis. Whereas the intensity and duration of PS exposure during viable cell activation depends on cell type and agonists, in apoptotic cells it constitutes a prerequisite for engulfment by phagocytes before any loss of plasma membrane integrity (Balasubramanian & Schroit 2003). Apoptotic bodies, the fragments of apoptotic cells (size > 1 µm) containing fragmented DNA, also expose PS and follow the same fate on a delayed time scale. In the vascular territory, exposed PS serves as a catalytic template or functional surface for the assembling of blood coagulation factor complexes, thus promoting *in situ* hemostasis, a physiological function of activated platelets and shed platelet MPs. As prime sensors of procoagulant stimuli, platelets are main contributors to MP circulating levels (Morel *et al.* 2008b). Platelet-derived MPs can thus be found at low levels in the circulation of healthy individuals probably as a result of low grade surveillance activation of the haemostatic system (Berckmans *et al.* 2001).

In vitro, the interaction of membrane-PS with coagulation factors is inhibited by its affinity ligand annexin V in presence of calcium (Gidon-Jeangirard *et al.* 1999). This property of annexin V is exploited experimentally and in clinical practice in the detection of MPs using various assays eventually combined with fluorescence labeling (flow cytometry, capture assays, fluorescence MP tracking).

Microparticles' identity unveil activated or suffering cells

Membrane glycoproteins distinctive of the parental cells are present on circulating MPs allowing thereby identification of their cellular origin. Antibodies directed against cell-specific antigenic determinants are used for this purpose in flow cytometry or antibody capture assays. Elevated circulating levels of distinct MPs are now considered as an indicator of either platelet, endothelial or leukocyte activation.

Identification of MP origin constitutes therefore a solid advantage to determination of their sole number and represents a robust parameter in systemic or inflammatory diseases (Chironi *et al.* 2006) or when associated to vascular complications like in diabetes (Sabatier *et al.* 2002a). Identification of MPs of practically any cell origin in plasma or other biological fluids (CSF, tears, exudates, Fig. 2) would become possible provided that antibodies directed against cell-specific antigenic determinants were available (Cook *et al.* 2001, Morel *et al.* 2008a). Detection of a distinct MP population would then be considered a direct message from a specific tissue undergoing activation or damage.

Haemostatic properties of blood microparticles

The procoagulant activity of platelet MPs was initially identified in the precipitate of platelet-free plasma obtained at high-speed centrifugation (30 000*g*, 120 min) (Chargaff & West 1946) and containing electron dense "platelet-dust" (Wolf 1967). These seminal discoveries

established the basis for the isolation of MPs from plasma and opened up a new avenue in thrombosis research culminating in the discovery that circulating tissue factor (TF), the cellular trigger of blood clotting, is mostly associated with circulating MPs (Giesen *et al.* 1999). It has also been suggested that platelets may recover TF present in raft of leukocyte-derived MPs (Rauch *et al.* 2000, Del Conde *et al.* 2005, Falati *et al.* 2003) and that endothelial MPs may stimulate the expression of TF by leukocytes (Sabatier *et al.* 2002b). Current knowledge suggests that coagulation factors VII and IX, once bound to membrane-PS in the presence of calcium, are activated by MP-borne TF thereby initiating the coagulation cascade leading to thrombin generation and *in fine* to the formation of a fibrin-platelet clot. Thus, TF-expressing MPs released by leukocytes upon soluble P-selectin stimulation enhance thrombus formation (Andre *et al.* 2000). P-selectin is a cell-adhesion molecule released by thrombin-activated platelets and endothelial cells into the circulation and its soluble form is a useful biomarker in ischemic events such as stroke (Nadar *et al.* 2004). In this regard, it was recently shown that mice producing abnormally high plasma levels of soluble P-selectin, had local uneven blood brain barrier disruption, silent brain infarctions and increased infarct size volumes in a experimental model of middle cerebral artery occlusion (Kisucka *et al.* 2009).

First clinical evidences of the haemostatic properties of procoagulant MPs were shown in patients with idiopathic thrombotic purpura (ITP see below) in whom high MP circulating levels were found protective against secondary hemorrhages. Conversely, in Scott syndrome, a very rare bleeding disorder, platelet PS exposure, membrane remodeling and MP shedding are defective and can be treated by platelet transfusion (Weiss 1994, Toti *et al.* 1996). Ultimate evidence of the haemostatic properties of MPs was given in engineered hemophiliac mice in which circulating leukocyte MPs correct hemostasis (Hrachovinova *et al.* 2003).

Most clinical studies have focused on the procoagulant role of platelet- and leukocyte-derived MPs as a determinant of the risk of cardio- and cerebro-vascular ischemic accidents as well as

in thrombotic associated disorders (Morel *et al.* 2004a, Simak *et al.* 2006, Chironi *et al.* 2009). However, it has recently been suggested that endothelial-derived MPs may also express anticoagulant or profibrinolytic properties, thereby complementing their procoagulant activity. The anticoagulant property of MPs is based, in part, on their ability to promote activation of protein C by thrombin, both assembled on their respective surface receptors thrombomodulin and endothelial protein C receptor (EPCR). Activated protein C (APC) bound to MP-EPCR inactivates procoagulant co-factors Va and VIIIa, thereby down-regulating thrombin generation (Satta *et al.* 1997, Perez-Casal *et al.* 2005). Endothelial MPs also express matrix metalloproteinases (EC 3.4.24) (Taraboletti *et al.* 2002) and MPs from the atherosclerotic plaque bear the TNF-α-converting enzyme (TACE) that are able to enhance endothelial cell surface processing of TNF-α and EPCR (Canault *et al.* 2007). The recent discovery of a profibrinolytic activity on MPs adds further to their contribution in the maintenance of vascular integrity. MPs shed by TNFα-stimulated endothelial cells, serve indeed as a surface for assembly of plasminogen and its conversion into plasmin (EC 3.4.21.7) by urokinase (uPA; EC 3.4.21.73) bound to its receptor (uPAR) (Lacroix *et al.* 2007). This capacity of endothelial MPs to promote plasmin generation confers them new profibrinolytic and, in concert with matrix metalloproteinases, proteolytic functions (Doeuvre & Angles-Cano 2009). The proteolytic activity of MPs may be of relevance in fibrinolysis, cell migration, angiogenesis, dissemination of malignant cells, cell detachment and apoptosis.

Beyond hemostasis: microparticles are dynamic pools of bioactive effectors

Apart from being membrane templates that harbor procoagulant, fibrinolytic and proteolytic factors as well as their distinctive glycoproteins, MPs may also carry molecular components (membrane receptors, cytokines, transcription factors, mRNA), veritable indicators of the activation status of the parental cell. MPs thus constitute a disseminated dynamic pool of

bioactive effectors or messengers, as documented by several *in vitro* studies (Ahn 2005, Morel et al. 2004c). Some of these MP components may exert *in situ* functions such as local fibrinolytic and proteolytic activities induced by uPA and metalloproteinases (Lacroix et al. 2007, Taraboletti et al. 2002, Graves *et al.* 2004). The intercellular transfer by MPs of mRNA (Deregibus *et al.* 2007, Ratajczak *et al.* 2006, Bruno *et al.* 2009) or membrane proteins like platelet glycoprotein GPIIb/IIIa to leukocytes (Salanova *et al.* 2007) or endothelial progenitor cell cultures (Prokopi *et al.* 2009), leukocyte TF to platelets (Falati et al. 2003) or the monocyte chemokine receptor 5 (CCR5) to endothelial cells (Mack *et al.* 2000), might have pathophysiological consequences for intercellular communication. MPs may also be conveyors of infectious agents delivered to target cells (human immuno deficiency virus, prions) (Simak *et al.* 2002) and of oncogens transferred from glioma MPs to naïve cells (Al-Nedawi *et al.* 2008). Despite difficulties in the assessment of membrane proteins, proteomic approaches combining two-dimensional electrophoresis and mass spectrophotometry, have expanded the number of identified proteins harbored by MPs of various origins (Miguet *et al.* 2006).

Microparticles are different from exosomes

The isolation of MPs follows a very precise protocol including a succession of various centrifugations at 20 000*g*. A consensus on a method of isolation has not been obtained as yet, but it appears clear that the relative gravitational force necessary to sediment MPs (15 000 to 20 000*g*, 45 to 90 min) is quite different from that used to isolate exosomes (as verified by light scattering measurement, authors' unpublished data). Exosomes are vesicles of endosomic origin, of smaller size (<100 nm) than MPs and are therefore isolated by sequential ultracentrifugation at very high speed (100 000*g*) (Thery *et al.* 2006). They are

secreted in the extracellular medium after fusion of multivesicular endosomes with the plasma membrane (for review, see (Thery *et al.* 2002)). Exosomes and MPs are biochemically and morphologically distinct, and have different patterns of protein composition (Thery *et al.* 2001). Exosomes are particularly enriched in tetraspanins, annexins and major histocompatibility complex class II molecules. Since membrane vesicles isolated at 50 000g to 100 000g may contain both exosomes and MPs, molecular components or pathophysiological involvement cannot be ascribed to the effect of single vesicles, however.

Effects of microparticles on inflammation and apoptosis

Vascular MPs behave as a dynamic storage pool of bioactive effectors able to tune the haemostatic balance, achieve vessel protection and complete restoration of blood flow. In addition, vascular MPs have been recognized as inflammatory actors via the transcellular delivery of bioactive lipids, chemokines (RANTES) or cytokines (IL-1 ß) (Barry *et al.* 1997, Mause *et al.* 2005, MacKenzie *et al.* 2001, Mesri & Altieri 1999). The question arises whether these transcellular cross-talk are all pathophysiological, *i.e.* deleterious (Freyssinet 2003, Morel *et al.* 2009), or as suggested by recent *in vitro* data, a beneficial effect may be expected. For instance, neutrophil-derived MPs were shown to inhibit the macrophage pro-inflammatory response to lipopolysaccharide and up-regulate macrophage TGF-ß secretion (Gasser & Schieferli 2004). Furthermore, MPs shed from adherent neutrophils convey annexin I, an anti-inflammatory protein able to inhibit further neutrophil adhesion thereby providing a negative regulatory loop to their recruitment at the inflamed endothelium (Dalli *et al.* 2008). Similarly, on endothelial cell cultures, early cytoprotection may also occur through the sorting of deleterious pro-apoptotic factors like caspase 3 in MPs, a mechanism that would prevent endothelial cell detachment and apoptosis (Abid Hussein *et al.* 2007). In a very recent

report, RNA-dependent apoptosis resistance and *in vitro* proliferation was conferred to tubular epithelial cells by MPs derived from human bone marrow mesenchimal stem cells (Bruno et al. 2009). Recovery of cultured rat oligodendrocytes from complement mediated attack through MPs shedding of membrane-attack complexes, may protect cells from complement-mediated lysis (Scolding *et al.* 1989, Pilzer *et al.* 2005). Other cytoprotective mechanisms rely on the MP-mediated modulation of apoptosis-related genes or pro-inflammatory cytokines, as recently shown in endothelial cells treated by APC-bearing MPs and confirmed in heatstroken baboons treated by recombinant human APC (Pérez-Casal *et al.* 2009, Bouchama *et al.* 2008). In addition, it has been recently suggested that MPs bearing EPCR would contribute to the cytoprotective effects of therapeutical APC, known to reduce mortality in sepsis and provide neuroprotective benefit in ischemic stroke (Soriano *et al.* 2005, Kerschen *et al.* 2007).

The adjustment between deleterious or beneficial responses to MP signals deserves extensive investigation and probably relies on multiple actors, including intracellular signaling kinases (Al-Nedawi et al. 2008, Schoenwaelder *et al.* 2009). New experimental approaches are needed to decipher the mechanisms governing the sorting out of beneficial or deleterious molecules into MPs and their relevance in distinct pathophysiological settings.

Current Analytical Methods

Because of the increasing importance of MPs as potential biomarkers, messengers or mediators of disease pathophysiology, particular attention has been given to pre-analytical sample conditioning and to biophysical methods for their detection and characterization (Jy *et al.* 2004a, Hugel *et al.* 2004). Appropriate blood collection to avoid artefactual cell activation and rigorous sample processing to isolate exosome-free MPs (see above) are indispensable.

Among the available detection methods, the most currently used is flow cytometry. It allows characterization and quantification of MP subpopulations in heterogeneous samples and may be directly used to analyse MPs in plasma samples (Robert *et al.* 2009). However, its main pitfall is that MPs with a size range below 500 nm (under the limit of the laser beam wave length) cannot be accurately detected. Therefore, quantitative analysis of MPs should be interpreted cautiously. An alternative capture assay using annexin V measures all procoagulant MPs bearing PS irrespective of size and origin. This functional assay provides quantitative results in terms of nmol/l of PS equivalent by comparison with a calibration curve constructed with synthetic vesicles containing known amounts of PS (Hugel et al. 2004). Annexin V-coated beads have also been used for MP visualization (Bianco *et al.* 2005). Newer methods for the measurement of MP size and distribution may be envisaged using their physical properties. Thus, photon correlation spectroscopy of back scattered light (Lawrie *et al.* 2009) and enhanced laser microscopy microparticle tracking (K Braeckmans, University of Ghent, personal communication) are emerging in the field of cellular MPs. The latter has the advantage of identifying the cellular origin of MPs with the use of specific fluorescent-labeled antibodies (Braeckmans K and authors' unpublished data).

Microparticles in central nervous system pathologies

The association of blood-derived MPs with a variety of inflammatory and/or prothrombotic states has been extensively studied (Morel *et al.* 2004b, Diamant *et al.* 2004, Distler *et al.* 2005, Pilzer et al. 2005, Leroyer *et al.* 2008, Morel et al. 2006). At present, all available reports on MPs in CNS or neurovascular pathologies have also focused on endothelial- or blood cells-derived MPs (Horstman *et al.* 2007). The survey of the literature we have made on MP involvement in CNS pathophysiology until April 28, 2009, concerns a limited number of

diseases as reported here. Although virtually any cell type may be constrain to release MPs, little is know about MP release from brain structures or cells of the neurovascular unit. Yet, the presence of galactocerebroside-containing MPs, suggesting oligodendrocyte origin, has been reported in CSF of patients with multiple sclerosis (MS) (Scolding et al. 1989) and *in vitro* studies indicate that MPs can be isolated from the supernatant of glial cells like astrocytes using annexin V-coated beads (Bianco et al. 2005, Bianco *et al.* 2009).

Ischemic stroke

A potential pathophysiological link between elevated concentration of platelet-derived MPs and development of cerebrovascular infarction was first reported in patients with prosthetic heart valves (Geiser *et al.* 1998). A limited number of prospective studies on MPs of endothelial and blood origin in ischemic stroke have then been reported. For instance, a significant high concentration of platelet-derived MPs was found in peripheral blood within 7 days and at 6 month follow-up of ischemic stroke (Cherian *et al.* 2003). This increase in platelet MPs after stroke was confirmed by other studies and was counteracted using a combination of the anti-platelet drugs aspirin and clopidogrel (Serebruany *et al.* 2005, Serebruany *et al.* 2008, Pawelczyk *et al.* 2009). The severity, lesion volume and outcome of acute ischemic stroke were also associated with an increased number of circulating endothelial-derived MPs (Simak et al. 2006). However, in all these reports, the prognostic value of these findings on recurrence of stroke and survival free of handicap could not be established. Furthermore, a comparison of endothelial MPs levels in acute ischemic stroke versus stroke mimic patients showed no difference (Williams *et al.* 2007). Since it is currently impossible to determine the anatomical origin of the endothelial MPs (systemic or neurovascular ischemic stimulation), it is difficult to ascertain whether MPs are epiphenomenal markers or active players in ischemic stroke.

Elevated levels of platelet MPs have been also observed in patients with transient ischemic attacks (TIA) and small vessels cerebro-vascular accidents including lacunar infarcts, and multiinfarct dementias (Lee *et al.* 1993, Geiser et al. 1998). Although in patients with TIA the number of platelet MPs may be importantly decreased under anti-platelet drug therapy, benefits for prevention of second stroke have not been reported (Serebruany et al. 2008).

Transient ischemic stroke in idiopathic thrombocytopenic purpura

ITP is an autoimmune disorder in which autoantibody-coated platelets are cleared by the mononuclear phagocytic system (Neylon *et al.* 2003). Since some of these autoantibodies bind to glycoproteins that induce platelet activation, platelet MPs are frequently elevated in these patients and thrombotic complications may develop despite severe thrombocytopenia and few signs of bleeding (Ahn *et al.* 2002). Patients with chronic late onset ITP (mean 56 years), may develop a syndrome characterized by neurological complications resembling transient cerebral ischemic attacks (TIA-like syndrome) and evolving from dizzy spells in mild cases to coma, seizure, or progressive memory loss and cognitive dysfunction in advanced cases (Jy *et al.* 1992). TIA-like syndrome in patients with ITP is indeed associated with magnetic resonance imaging findings (periventricular and subcortical white matter lesions) consistent with ischemic small vessels disease that may be a consequence of platelet MP-induced microthrombi (Ahn et al. 2002). Recent *in vitro* data suggest that the release of endothelial MPs and the extravasation of leukocytes may also contribute to development of the ischemic brain disease (Jimenez *et al.* 2008). These data suggest that ITP and ischemic stroke are not mutually exclusive events (Theeler & Ney 2008). If proper identification of this syndrome (e.g. measurement of MPs) is made, therapy could then be targeted at prevention of thrombotic complication rather than hemorrhages. Indeed, ITP patients, with elevated MP levels were shown to be at lower risk of hemorrhages, probably because procoagulant MPs

behave as alternate procoagulant catalytic surfaces under circumstances of cytopenia (Jy et al. 1992).

Microparticles in cerebrospinal fluid

Recently, it was shown that the plasma and the CSF of patients suffering from traumatic brain injury (Morel et al. 2008a) contain PS exposing MPs mainly of platelet and endothelial origin. Procoagulant MPs were also found significantly elevated in the CSF of patients with hemorrhagic stroke as detected by annexin V binding/procoagulant assay (Huang *et al.* 2009). The sustained generation of these procoagulant MPs in the CSF of some patients could contribute to a poor clinical outcome. MPs (0.1 to 0.5 μm in diameter) reactive with antibodies to complement membrane-attack complex neoantigen and galactocerebroside were identified in CSF of multiple sclerosis patients by electron microscopy (Scolding et al. 1989) suggesting that reversible complement-mediated injury contributes to myelin damage *in vivo*. In patients with glioblastoma, membrane vesicles were identified in CSF after sequential centrifugation at 10 000g and 200 000*g* (Huttner *et al.* 2008), a procedure frequently used to separate exosomes. These membrane particles (previously identified as 50-80 nm particles (Marzesco *et al.* 2005)), contained the neural stem cell marker prominin-1/CD133, but neither PS nor cell identity antigens were reported. Prominin-1/CD133 is a marker that decline postnatally until 10 years of age and was found elevated in glioblastoma patients. A proteomic analysis of human embryo CSF revealed a heterogeneous mixture of functionally diverse proteins including proteins with extracellular matrix functions, secreted proteases and their inhibitors, and cell adhesion proteins (Zappaterra *et al.* 2007). Interestingly, the presence of membrane proteins, signaling molecules and other intracellular proteins are most likely of MP and/or exosome origin that have been previously described in CSF (Marzesco et al. 2005, Scolding et al. 1989).

Cerebral Malaria

Circulating endothelial-derived MPs are increased in patients with severe cerebral malaria (1 to 8 % of *Plasmodium falciparum* infections) complicated with coma as compared with uncomplicated malaria or healthy control (Combes *et al.* 2004). Parasite-derived products activate platelets and promote monocyte TNF-α production, a well-known inducer of endothelial MPs *in vitro*. Binding of activated platelets to TNF-α-primed endothelial cells would lead to platelet adhesion and blood clogging, and the release of MPs within brain microvasculature with subsequent induction of permeability changes, ischemia, endothelial cell apoptosis and cerebral oedema (van der Heyde *et al.* 2006).

Combes *et al.* provided major insights in the mechanism of action of MPs in cerebral malaria (Combes *et al.* 2005). These authors reported that in a mouse model, ABCA1, a membrane transporter that mediates cholesterol translocation and a casual floppase known to facilitate the transbilayer distribution of PS to the outer leaflet of the membrane (Fig. 1), might contribute to cerebral malaria via MP shedding. Indeed, external exposure of PS is impaired in ABC-1 knockout mice that also show low circulating MP levels and a complete resistance to cerebral malaria (ablated platelet accumulation in brain microvessels). These data suggest, but do not prove, that endothelial MPs are directly implicated in the mechanism of human cerebral malaria. Interestingly, some biological manifestations (impaired PS exposure and defective vesiculation by EBV-lymphocytes) of a patient with impaired plasma membrane expression of ABCA1 are found in patients with Scott syndrome and normal ABCA1 (Albrecht *et al.* 2005, Toti & Freyssinet 2005).

Multiple sclerosis

MS is characterized by the presence of inflammatory white and gray matter lesions in the brain and spinal cord (Frohman *et al.* 2006). Demyelination and oligodendrocyte degeneration are hallmarks in MS. Oligodendrocytes activated by inflammatory cells recover from cell injury via the release of MPs enriched in complement membrane-attack complexes (Scolding et al. 1989). Such oligodendrocyte-derived MPs have been found in the CSF of MS patients thus underlining their pathophysiological relevance (Scolding et al. 1989). Activation of leukocytes adhering to the neurovascular endothelium and their release of inflammatory cytokines (IFN-γ, TNF-α) that in turn activate the endothelium is thought to be another crucial step in the formation of demyelinating lesions. As a consequence, elevated levels of circulating MPs have been documented in MS. They reflect endothelial dysfunction induced by the inflammatory cytokines and could be associated with a poor clinical outcome (Minagar & Alexander 2003). High plasma levels of endothelial MPs carrying CD31 (PECAM-1, platelet endothelial cell adhesion molecule) were detected during disease exacerbation and returned to nearly control value during remission (Minagar *et al.* 2001). The presence of such MPs was in positive association with contrast-enhancing lesions by brain magnetic resonance imaging. These authors suggested that a high rate of CD31-endothelial MPs in plasma would rather be a marker of exacerbation (acute injury of endothelium) while endothelial MPs carrying CD51 (vitronectin receptor) may reflect MS relapse (chronic injury of endothelium). These markers reflecting the state of the endothelium are not distinctive of MS, but can be of help in follow-up, once the diagnosis of MS has been established. For instance, treatment of relapsing-remitting MS patients with interferon-β1a significantly reduced plasma levels of CD31-endothelial MPs (Sheremata *et al.* 2006). In the absence of any specific probe, the ratio of endothelial MPs carrying CD54 (ICAM-1, intercellular adhesion molecule-1) to monocyte number was proposed as a better parameter of MS since it was found increased during the acute phase compared to remission or healthy controls (Jy *et al.* 2004b). The value of this

ratio has also been used to appreciate the response to interferon-β1b treatment (Sheremata et al. 2006). In an *in vitro* study, it has indeed been shown that interferon-β1b partially inhibits MS plasma-induced endothelial MP formation as well as the transmigration of monocytes or monocytes/endothelial MP complexes (Jimenez *et al.* 2005).

Experimental studies

In vivo murine experiments have shown that cancer cell-derived MPs may be involved in the propagation of oncogenes. For instance, the membrane oncogenic epidermal growth factor receptor EGFRvIII can be exchanged between cultured U373 glioma cells by a PS-dependent intercellular transfer of MPs. Incorporation of EGFRvIII into the U373 plasma membrane resulted in a consistent increase in Erk1/2 phosphorylation. Furthermore, subcutaneously injected tumor cells into immunodeficient mice causes extracellular and systemic release of microvesicles carrying the EGFRvIII oncoprotein, suggesting that these MPs may serve as vehicles for rapid intercellular transfer of the transforming activity between cells populating brain tumors (Al-Nedawi et al. 2008). The horizontal transfer (without cell-cell contact) of this receptor to naïve cells may contribute to propagation of oncogenes and their associated transforming phenotype. Such a mechanism may be operative in a variety of human brain tumors and disseminates *via* blood to distant sites.

Conclusion and perspectives

Because the plasma membrane is the primary sensor of cell interactions with the microenvironment, plasma membrane remodeling including PS exposure and release of MPs is a characteristic feature of blood/vascular cell response to different type of stimuli. The identity of circulating MPs of endothelial, platelet and leukocyte origin is indeed a reliable indicator of their activation in CNS diseases such as stroke, TIA, cerebral malaria and MS.

The most characterized property of MPs is their procoagulant activity and a number of studies have established a clear relationship with thrombosis development in cardiovascular and cerebro-vascular ischemic diseases. By virtue of the increasing number of biomolecules identified on/in MPs, cell-derived MPs are emerging as mediators of intercellular communication and new messengers/biomarkers from tissues undergoing activation or damage. They could be reliable markers of CNS pathophysiological processes useful in biomedical research and clinical medicine. For that purpose, efforts should be made to develop new biological tools and methods able to detect brain/neurovascular tissue-specific MPs. This challenging approach may open new perspectives and developments in the field of neuroscience, particularly in pathologies, such as Alzheimer's disease, still virgin to MP detection.

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Table 1. Cell-derived microparticles in CNS diseases.

Pathology	Compartment	MP pattern	Technique used	Reference
Stroke	Blood	Elevated platelets-derived MPs Elevated platelets-derived MPs Association of endothelial MPs with lesion	Flow Cytometry	Cherian et al. 2003 Pawelczyl et al. 2009 Simak et al. 2006
Transient ischemic stroke		Elevated platelets-derived MPs		Lee et al. 1993
Malaria		Elevated endothelial-derived MPs		Combes et al. 2004
Multiple Sclerosis	Plasma	High level of endothelial MPs		*Jy et al. 2004*
	CSF	Presence of oligodendrocyte-derived MPs	Electron microscopy	Scolding et al. 1989
Traumatic Brain Injury	Plasma and CSF	Presence of platelet and endothelial MPs	Prothrombinase assay	Morel et al. 2008
Glioblastoma	CSF	Presence of platelet and endothelial MPs	Immunoblot	Huttner et al. 2008

FIGURE LEGENDS

Figure 1. Blebbling and shedding of membrane microparticles during cell activation.

Cell membrane phospholipid asymmetry results from the concerted activity of lipid transporters responsible for their inward (flippases), outward (floppases) or bidirectional (scramblases) translocation. Accordingly, aminophospholipids, mainly phosphatidylserine (polar head blue) and phosphatidylethanolamine are sequestered in the internal leaflet of the plasma membrane. Upon cell activation and calcium influx, scramblase activity overwhelm flippase activity and phosphatidylserine is rapidly translocated to the external membrane leaflet. Cleavage of the cytoskeleton promotes budding of the stimulated cell membrane and shedding of microparticles. Formation of transient membrane cholesterol-rich microdomains termed raft may provide an appropriate platform for the assembly of regulatory elements and cell agonists, and the initiation of transmembrane signaling pathways. Shed microparticles bear cell-specific proteins (CD xx) as well as bioactive molecular components (e.g. growth factors, proteolytic enzymes, mRNA…) from the parental cell cytoplasm and from the plasma membrane.

Figure 2. Microparticles : new biomarkers in central nervous system pathology.

Cells in the neurovascular unit are subjected to activation by different types of stimuli (e.g. oxydants, inflammation and ischemia). As a consequence, activated cells release membrane microparticles (Fig. 1). Microparticles carry identity proteins and bioactive molecules from the parental cell. Their detection and identification in blood, CSF and other body fluids (e.g. tears, nasal mucus) would then be considered as a direct indicator of activation or damage from specific cells or tissues.

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