Platelets and neurovascular inflammation

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Summary
Platelets participate in haemostasis and in thrombus formation in health and disease. Moreover, they contribute to inflammation and cooperate with immune cells in a magnitude of inflammatory/immune responses. Although the inflammatory response has been recognised to be critical in neuronal diseases such as Alzheimer’s disease or multiple sclerosis its major role in the brain is poorly investigated so far. Emerging studies, however, point to an interesting crosstalk between platelets and neuroinflammation. For instance, when the integrity of the blood brain barrier is compromised, platelets may be relevant for endothelial inflammation, as well as recruitment and activation of inflammatory cells, thereby potentially contributing to central nervous tissue pathogenesis. This review summarises recent insights in the role of platelets for neurovascular inflammation and addresses potential underlying mechanisms, by which platelets may affect the pathophysiology of neurovascular diseases.

Platelets and inflammation
Platelets are of major importance in haemostasis. However, significant evidence in recent years indicates that platelets are crucial players in inflammatory and immune responses. Due to their intimate crosstalk with leukocytes, platelets could be considered as an extended arm of innate immunity. In the course of vascular inflammation or injury, platelets associated with the endothelium or the subendothelial space serve to promote inflammatory cell recruitment, thereby orchestrating the inflammatory response; this may be of particular importance in atherosclerotic disease processes (1-4).

Inflammatory cells, such as neutrophils or monocytes can interact with endothelium-adherent platelets; thereby platelets can mediate the adhesion of inflammatory cells to the inflamed vessel wall. The adhesive interactions that contribute to the cross-talk between leukocytes and platelets include: (i) The binding of P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes to platelet P-selectin (5). (ii) The interactions between the leukocyte beta2-integrin Mac-1 and glycoprotein Ib (GPIb) or junctional adhesion molecule-C on platelets, whereby the former interaction promotes leukocyte-platelet adhesion under both high and low shear stress, and the latter interaction primarily operates under low shear stress conditions (6-9). Alternatively, fibrinogen bound to GPIIb/IIIa on platelets can also interact with leukocyte Mac-1 thereby forming a bridge between platelets and neutrophils; this interaction can be triggered by platelet activating factor (10). Through these interactions platelets have been implicated in several inflammatory conditions (11). In particular, blocking platelets or the Mac-1/GPIb interaction has proven to be a promising therapeutic approach in animal models of vascular injury, vasculitis, glomerulonephritis and more recently, neuroinflammation (11-13). Moreover, platelets have been implicated as orchestrators of the inflammatory response in arthritis (14) or sepsis; in the latter scenario, platelets trigger formation of neutrophil extracellular traps in the defence against bacterial infection (15). Besides mediating inflammatory cell recruitment, platelets are also a major source of chemokines and cytokines at the site of inflammation (2). Another mechanism, by which platelets may promote inflammation, is via platelet-derived microparticles. Similar to endothelial cells and leukocytes, platelets can release microparticles, which have been implicated in endothelial and tissue inflammation (16-22).

Here, we will focus on recent evidence with regards to the role of platelets in neuroinflammation. Whereas we will not discuss the role of platelets in atherosclerotic conditions of the central nervous system, we will focus briefly on the role of platelets and thromboinflammation in stroke and will review and discuss the function of platelets in diseases, such as Alzheimer’s disease or multiple sclerosis in detail.

In the course of neurovascular inflammation, a unique component is the brain microvasculature, which significantly differs from other vascular beds (Figure 1). Not only endothelial cells or microglia cells are relevant for brain inflammation (23-26), platelets also participate in tumour necrosis factor (TNF)-induced brain inflammation. Depletion of platelets significantly reduced leukocyte rolling and adhesion on the inflamed endothelium in...
the course of TNF-induced brain inflammation (27). Whereas endothelial P-selectin and E-selectin played a role in TNF-induced brain inflammation, platelet P-selectin was not involved in this process, suggesting that other platelet receptors may mediate inflammatory cell recruitment in this scenario (27). Intravital imaging studies in an animal model of murine cerebral malaria using microparticles of iron oxide conjugated to an antibody specific for activated platelet GPIIb/IIIa receptor demonstrated the presence of activated platelets in the inflamed brain, which suggests the possibility to use platelet associated molecules for diagnostic purposes in the setting of neuroinflammation (28). Interestingly, in this study TNF, but not other cytokines, induced interaction of platelets with the brain endothelium (28). These data are strengthened by in vitro studies demonstrating that platelets can stimulate a proinflammatory expression profile in brain microvascular endothelial cells in the context of cerebral malaria (29). Platelets themselves can release several cytokines that contribute to endothelial inflammation (30-32). A recent study demonstrated that murine platelets express interleukin-1α (IL-1α), and that platelets enhanced the expression of adhesion molecules intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 in cultured brain endothelial cells, but also induced the release of CXCL1 chemokine (33). Furthermore, this study demonstrated that the presence of platelets in the inflamed central nervous tissue correlated with activation of the endothelium (33).

Although further studies in animal models of neurovascular inflammation are required, available data to date indicate that the interplay of platelets and platelet-derived effectors with brain endothelium and leukocytes may modulate the course of neuroinflammatory diseases, such as stroke, Alzheimer’s disease and multiple sclerosis or its mouse counterpart, experimental autoimmune encephalomyelitis (EAE).

Platelets and stroke

Most strokes are caused by thrombotic or embolic vessel occlusions. The role of platelets in stroke is unabated and has been reviewed extensively elsewhere (34, 35). Intriguingly, only recently has the inflammatory role of platelets in stroke been appreciated (34). The introduction of appropriate mouse models with genetic deletion of specific platelet receptors and platelet proteins has triggered emerging studies on the mechanisms, by which platelets can contribute to thrombin formation and regeneration in stroke (34, 36-39). Inhibition of platelet adhesion to the injured vessel wall by blocking platelet surface receptors, such as GPIIba or GPVIIb are protected from stroke without necessarily provoking bleeding complications (36, 40, 41). Furthermore, signalling events downstream of adhesion receptors GPIIIa and GPVIIb participate in the pathophysiology of stroke as well (42). In addition, the relevance of platelet microparticles in this disease setting has been proposed (43). Previously, endothelial microparticles were correlated with disease severity and the size of the lesion in the course of acute ischaemic stroke (44). More recently, Lukasik et al. could demonstrate that the presence of platelet microparticles may positively correlate with the convalescence from stroke (45). In the same study, a negative correlation between the percentage of platelet-derived microparticles and the expression of the platelet activation marker P-Selectin was also found (45). Future studies are required to further evaluate the functional role of platelet microparticles for the pathophysiology of stroke.

Platelets and Alzheimer’s disease

Alzheimer’s disease, being the most common course of dementia, has attracted a lot of scientific attention in recent studies aiming at understanding its underlying pathology (46). Alzheimer’s disease pathophysiology comprises a complex network including inflammation, deposition of β-amyloid and neuronal loss, altogether culminating in brain dysfunction (47, 48). Although efforts to understand this disease range from structural biology (49) to elegant mouse models (50) to clinical trials for treatment of disease symptoms (51), the progress in targeting the underlying cause of the disease is rather slow, which could be explained by the complexity of the processes contributing to disease initiation and progression. Deterioration of memory and neuronal function is associated with the accumulation of misfolded proteins in the ageing brain, including phenomena such as amyloid precursor protein proteolysis and self-aggregation (47, 48), tau protein deposition and neurodegeneration (48, 52).

Emerging evidence points to an important role of vascular inflammation and the breakdown of the blood brain barrier (BBB) in Alzheimer’s disease (47). As a component of vascular inflammation (1, 4), platelets are likely to be involved in diseases affecting integrity of the BBB. Altered platelet function in Alzheimer’s disease has been reported in several studies for over 10 years now (53-55). The fact that platelets contain substantial amounts of amyloid precursor protein (APP) has resulted into the hypothesis that platelets contribute to Alzheimer’s disease (56). Activated platelets may mediate processing of amyloid precursor protein into β-amyloid peptide and its release (57), which may promote the deposition of amyloid around the vasculature (56, 57). Several studies have suggested platelet associated proteins have diagnostic value in Alzheimer’s disease. Zainaghi et al. demonstrated that a decrease in the platelet amyloid precursor protein ratio can serve as a predictive factor for the progression of mild cognitive impairment to Alzheimer’s disease (58), whereas Vignini et al. suggested that different platelet amyloid precursor protein isoforms could serve as potential peripheral markers in Alzheimer’s disease (59). Moreover, it has been shown that platelet activation or the level of soluble GPVIIb may be of predictive value in Alzheimer’s disease (60, 61). Interestingly, human platelets are a source of various forms of tau protein, which could be of diagnostic or pathophysiological relevance (62). A common denominator between Alzheimer’s disease and other diseases involving platelet activation, such as atherosclerosis is protein misfolding (63). Notably, protein misfolding can induce platelet aggregation, a process which involves CD36, p38(MAPK), as well as GPIIb-IIIa-induced aggregation (64).
Platelets express PPAR α, β/δ and γ, and their ligation blunts the release of proinflammatory mediators from platelets (65, 66); interestingly, in Alzheimer’s disease manipulation of PPARs is considered as a potential therapeutic approach (67). Given the amount of data indicating a potential relevance of platelets for Alzheimer’s disease it is not surprising that first interventions targeting platelets are underway. These trials, however, have not been successful so far (68, 69). Targeting platelets in Alzheimer’s should aim at preventing their proinflammatory/prothrombotic actions, while not affecting their haemostatic function.

Platelets and multiple sclerosis

Multiple sclerosis (MS) is a devastating neuro-inflammatory disease. An autoimmune reaction is thought to trigger MS. In the rodent model of the disease, EAE, animals are immunised with antigens derived from the myelin sheath (e.g. myelin oligodendrocyte glycoprotein or myelin basic protein), which results in infiltration of autoimmune T cells into the brain and spinal cord. This autoimmune reaction induces a further wave of inflammatory cell (neutrophils, monocytes, macrophages) recruitment along with the pro-inflammatory stimulation of microglia cells, altogether resulting in destruction of the myelin sheath, which precipitates demyelinating lesions (70, 71). The importance of inflammatory cell recruitment into the inflamed central nervous tissue in MS and EAE pathogenesis is underscored by animal studies with inhibition / genetic ablation of leukocyte or endothelial adhesion molecules (72-75). In addition, blocking antibodies against leukocyte integrin VLA-4 represent a potent therapeutic approach in human MS (76).

Prompted by findings derived from a microarray analysis of human MS plaques that identified increased levels of the platelet receptor αIIb-integrin (GPIIb) mRNA in chronic lesions of MS patients (77), as well as by studies demonstrating that platelets are present in the murine brain in the course of EAE (78), we recently assessed the role of platelets in EAE. We found that platelets clearly contributed to EAE disease pathogenesis, and thus, potentially to human MS (12) (Figure 1). We found that platelets were abundant in the inflamed brain and spinal cord of mice subjected to the

**Figure 1:** Under physiological conditions, the blood brain barrier (BBB) is tight and prevents adhesion and penetration of inflammatory cells. Upon inflammatory stimulation, e.g. in multiple sclerosis, the barrier itself and the anti-inflammatory function of the BBB are compromised. As a consequence, inflammatory cells such as T cells (T) or monocytes / macrophages (MΦ) penetrate the BBB and resident inflammatory cells such as microglia cells (mic) are activated. Besides inflammatory cells, platelets adhere to the endothelium and contribute to neurovascular inflammation through multiple interactions with inflammatory and endothelial cells. The insert depicts receptor/ligand pairs that mediate inflammatory cell and endothelial cell interaction with platelets.
Figure 2: Contribution of platelets to neuroinflammation. Platelets, which are present in the inflamed neuronal tissue, may contribute to neurovascular inflammation and neuronal disease in several ways. Similarly to other pathologies with endothelial inflammation, platelets can adhere to the endothelium and propagate inflammation. This may occur through several mechanisms including leukocyte recruitment, activation of inflammatory cells including microglia cells, aggravation of endothelial inflammation and release of pro-inflammatory mediators such as cytokines or chemokines.

EAE model. These data were supported by histological analysis of chronic active lesions of MS patients, in which platelets were present (12). Platelet depletion in the effector phase of the disease resulted in significant amelioration of EAE disease severity, associated with reduced demyelination and axonal damage. In contrast, platelet depletion in the immunisation phase of EAE did not influence EAE development. These data suggested that platelets rather contribute to the inflammatory phase of the disease (12). Consistently, platelet depletion resulted in a blunted inflammatory response in the course of EAE, including reduced numbers of recruited leukocytes into the inflamed spinal cord, as well as decreased levels of inflammatory cytokines and chemokines. Mechanistic experiments with intravital microscopy analysis revealed that platelets directly contributed to leukocyte rolling and adhesion onto the endothelium of the inflamed postcapillary venules of the inflamed central nervous tissue (12). Thus, platelets may exacerbate EAE inflammation at least in parts by mediating inflammatory cell adhesion to endothelial cells of the central nervous tissue. This observation led us to interfere with platelet adhesion receptors in order to inhibit platelet accumulation in the inflamed vessels of the central nervous tissue. Indeed, antibodies targeting GPIba or GPIb/IIIa suppressed EAE disease severity in mice (12). Moreover, using antibodies specifically targeting the interaction of GPIba with leukocyte Mac-1 significantly reduced EAE in mice (12). Previous findings have shown that inhibition of Mac-1 ameliorated EAE disease severity (79). In contrast, P-selectin did not seem to participate in murine EAE disease development (78), suggesting that GPIb-dependent but not P-selectin-dependent platelet interactions contribute to inflammation in the course of EAE. Consistent with our findings, MS patients displayed higher levels of platelet activation markers in their peripheral blood (80). Interestingly, platelets may be only one aspect of the interplay of the coagulatory and thrombo-inflammatory systems with central nervous tissue inflammation. In particular, tissue factor is one of the most abundant proteins in chronic active lesions of MS patients (81). Thus, we are only at the beginning of our understanding how platelets and further thrombo-inflammatory components may contribute to neuroinflammation, which is a field that merits future investigation.

Conclusions and future directions

Emerging evidence indicates that we need to broaden our view on the function of platelets. Platelets are not simply a cell of haemostasis/thrombosis but can unequivocally contribute to inflammatory processes (1, 2). Platelets should be, thus, considered cells of the innate immunity (1, 2). Recent experimental insights suggest that platelets clearly participate in the pathogenesis of neuroinflammatory diseases such as Alzheimer’s disease or multiple sclerosis (82) through multiple mechanisms (Figure 2). Future studies are required to assess the exact mechanism underlying the role of platelets in neuroinflammation. In addition, the function of platelets in further neuronal disorders, such as Parkinson’s disease or amyotrophic lateral sclerosis should be addressed. Such future studies will also establish, whether platelets might represent an attractive therapeutic target in neuroinflammation.

Acknowledgement

The work was supported by the Novartis Foundation for Therapeutic Research (to TC). Furthermore, this work was supported by the Volkswagen Foundation (Lichtenberg program), the Tuebingen Platelet Investigative Consortium (TuePIC) and the Clinical research unit 274 supported by the German Research Foundation, and the Wilhelm Sander Foundation (to H. F. L.).

Conflicts of interest

None declared.
Platelets: basic mechanisms and translational implications


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