Platelets and vascular inflammation of the brain

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Summary There is emerging evidence that platelets have an important role in inflammation beyond their involvement in hemostasis. Platelets can contribute to inflammatory reactions via crosstalk both with immune cells and endothelial cells. Inflamed vessels are characterized by the presence of activated endothelial cells. These activated endothelial cells upregulate receptors necessary for leukocyte recruitment, but also for the adhesion of platelets. Subsequently, immune cells can bind to platelets through adhesion receptors presented on the platelet surface, thus supporting leukocyte recruitment to the vessel wall. There are several neurological diseases associated with vascular inflammation including multiple sclerosis (MS) and stroke. Increased markers of platelet activation could be demonstrated in patients suffering from MS compared to healthy individuals. Reports from murine models indicate that platelets may be of importance for disease progression and severity by mediating leukocyte recruitment as one potential underlying mechanism. Blocking platelet function disease severity was considerably ameliorated. Moreover, processes of tissue remodelling may be influenced by platelet derived mediators. Whether a role of platelets for vascular inflammation can be extrapolated to further neurological diseases will have to be investigated in further depth experimental and clinical trials. Conclusion: Platelets and platelet associated mechanisms may offer novel starting points to understand neurovascular diseases from a different point of view and to develop novel approaches to access the disease.

Schlüsselwörter
Thrombozyten, Entzündung


The fact that platelets are strongly involved in vascular diseases is well known from the pathogenesis of coronary artery disease. Today, modulation of platelet function is central to acute treatment but also important for secondary prevention after myocardial infarction (1).

Platelets contribute not only to acute symptoms of infarction but also to the pathogenesis of atherosclerosis via interactions with immune cells and endothelial cells. Their atheromodulative effect is mediated by surface receptors and ligands as well as through soluble mediators directly released from platelets (2, 3).

Emerging evidence points to a detrimental role of platelets not only for coronary artery disease but also for neurological disorders featuring a strong neuroinflam-
Platelets, ligands, receptors

The mechanisms of hemostasis and thrombosis require a close interplay between platelets, the endothelium, plasmatic coagulation factors and structures of the vessel wall including the extracellular matrix (Fig. 1). Recently, platelets have also been implicated in inflammatory processes, and platelet adhesion receptors are major determinants of platelet-mediated inflammation. Platelets express glykoproteins on their membranes that mediate the interactions of platelets among themselves (GPIIb-IIIa) as well as with the subendothelial matrix (von Willebrand factor (VWF) receptors, collagen receptors), with plasmatic coagulation factors (VWF), and with endothelial cells (GPIIb-IIIa) or leukocytes (P-selectin).

Platelet adhesion receptors are classified into four groups according to their characteristic molecular structures: integrins, leukocyte-rich glycoproteins, selectins, and receptors of the immunoglobulin type. Major platelet adhesion receptors and their counterligands relevant for inflammation are summarized (Tab. 1).

**Integrins**

The integrins involved in vascular inflammation can be subdivided into

- $\beta_1$ ($\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$) and
- $\beta_3$ ($\alpha_5\beta_3$, $\alpha_6\beta_3$) subtypes.

Integrin $\alpha_5\beta_3$ is also known as glycoprotein GPIIIa, the fibrinogen receptor. For interaction with the extracellular matrix, integrins $\alpha_5\beta_3$ and $\alpha_6\beta_3$ are of particular relevance under in vivo conditions, and $\beta_3$ null mice treated with a blocking antibody against GPIIIa show an additional reduction in firm platelet adhesion after carotid endothelial injury compared to mice where only GPIIb-IIIa is blocked (10).

Integrin $\alpha_6\beta_3$ is important for binding of platelets to collagen, as platelets after in...
hinition of cyclooxygenase, P<sub>2</sub>Y<sub>1</sub>, P<sub>2</sub>Y<sub>12</sub> and GPIIb-IIIa show significantly reduced adhesion to collagen when α<sub>IIb</sub>β<sub>3</sub> is blocked with monoclonal antibodies (mAb) (11). Platelet spreading but not adhesion following α<sub>IIb</sub>β<sub>3</sub> activation is dependent on Src kinase because inhibition of Src kinase has an inhibitory effect on spreading of platelets whereas adhesion is not affected (11).

Also β<sub>3</sub> integrins on endothelial cells are relevant for platelet interaction with the vascular wall, particularly integrin α<sub>IIb</sub>β<sub>3</sub>, which is upregulated by interleukin 1 or thrombin (12). Interfering with integrin α<sub>IIb</sub>β<sub>3</sub> function leads to significantly less platelet adhesion on activated endothelium (12). GPIIb-IIIa is also relevant for platelet leukocyte interactions, because adherent platelets can recruit leukocytes (PMN) by interaction of MAC-1 on leukocytes with GPIIb-IIIa on platelets (13). Additionally, interactions of GPIIb-IIIa on platelets with fibrinogen and VWF were reported (14). Blockade of platelet bound VWF, fibrinogen and fibronectin with mAbs after stimulation with thrombin led to significantly decreased adhesion to human umbilical vascular endothelial cells (HUVEC) (14).

**Glycoproteins**

Another group of platelet adhesion receptors are the glycoproteins. Under conditions of vascular inflammation, GPIIbα as well as GPVI are of importance. GPIIbα is known to interact with VWF (15, 16). When GPIIbα is blocked with a mAb, atherosclerotic lesion development in ApoE<sup>−/−</sup> mice is significantly reduced (16). Both transient and firm platelet adhesion to the vessel wall is significantly diminished when GPIIbα is blocked with a mAb in ApoE<sup>−/−</sup> mice leading to reduced leukocyte adhesion to the vascular wall (16). Furthermore, it was demonstrated that impairment of both GPIIbα and GPVI function resulted in significantly decreased platelet tethering and significantly reduced firm adhesion of platelets in a mouse model of carotid artery injury (17). The direct platelet collagen interactions through platelet GPVI and collagen present in the subendothelial matrix of the vascular wall are essential not only for platelet tethering to collagen, but also for firm adhesion of platelets (17). Besides integrins and glycoproteins platelets can also interact with leukocytes and endothelial cells by selectins, for example by an interaction of P-selectin glycoprotein ligand 1 (PSGL-1) on platelets with P-selectin on endothelial cells (18).

Another possibility is the interaction of P-selectin on platelets with PSGL-1 on leukocytes or activated endothelial cells (19).

**Receptors of the immunoglobulin superfamily, chemokine receptors**

Finally, receptors of the immunoglobulin superfamily are important for platelet interactions. For instance, platelet leukocyte interactions depend on binding of ICAM-2 on platelets to LFA-1 on leukocytes (13, 20). This interaction mainly takes place when shear rates are low (13). Interestingly, the chemokine fractalkine (CX3CL1) on endothelial cells can interact in vitro with the fractalkine receptor CX3CR1 on platelets resulting in increased P-selectin expression on platelets (21). This is important for platelet P-selectin mediated leukocyte recruitment after platelet activation by fractalkine (21). Dendritic cells (DCs) representing a subset of leukocytes also show interactions with platelets because they can bind via MAC-1 on DCs to JAM-C on platelets under in vitro flow chamber conditions leading to firm adhesion of DCs on immobilized platelets (22). Recently, it was shown that JAM-A on platelets has an atheroprotective role in ApoE<sup>−/−</sup> mice as mice lacking JAM-A show increased platelet reactivity in vitro and increased atherosclerotic plaque formation especially in early stages of atherosclerosis pointing out an important role of this platelet adhesion receptor (23). In flow chamber experiments, platelets from ApoE<sup>−/−</sup> mice without JAM-A showed increased thrombus formation (23). In vivo, platelets from ApoE<sup>−/−</sup> mice lacking JAM-A can recruit more leukocytes in a model of carotid en-

### Table 1: Platelet receptors, platelet derived factors, platelet associated factors and their target structures contributing to vascular inflammation and neurovascular inflammation

<table>
<thead>
<tr>
<th>platelet receptors</th>
<th>ligand</th>
<th>interaction with</th>
<th>ref.</th>
</tr>
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<tbody>
<tr>
<td>integrins</td>
<td>α&lt;sub&gt;5&lt;/sub&gt;β&lt;sub&gt;1&lt;/sub&gt;, α&lt;sub&gt;6&lt;/sub&gt;β&lt;sub&gt;1&lt;/sub&gt;</td>
<td>subendothelial extracellular matrix</td>
<td>damaged vessel wall</td>
</tr>
<tr>
<td></td>
<td>α&lt;sub&gt;2&lt;/sub&gt;β&lt;sub&gt;1&lt;/sub&gt;</td>
<td>collagen</td>
<td>damaged vessel wall</td>
</tr>
<tr>
<td>glycoproteins</td>
<td>GPIIbα</td>
<td>VWF</td>
<td>damaged vessel wall</td>
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<tr>
<td></td>
<td>GPIIb/IIIa (αIIbβ3)</td>
<td>MAC-1</td>
<td>leukocytes</td>
</tr>
<tr>
<td></td>
<td>GPIIb/IIIa (αIIbβ3)</td>
<td>fibrinogen, VWF</td>
<td>vessel wall</td>
</tr>
<tr>
<td></td>
<td>GPIIb/IIIa (αIIbβ3)</td>
<td>fibrinogen, vitronectin</td>
<td>integrin αβ3 on endothelial cells</td>
</tr>
<tr>
<td></td>
<td>GPVI</td>
<td>collagen</td>
<td>damaged vessel wall</td>
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<tr>
<td>selectins</td>
<td>PSGL-1</td>
<td>P-selectin</td>
<td>endothelial cells</td>
</tr>
<tr>
<td></td>
<td>PSGL-1</td>
<td>P-selectin</td>
<td>leukocytes</td>
</tr>
<tr>
<td>others</td>
<td>CX3CR1</td>
<td>CX3CL1</td>
<td>endothelial cells</td>
</tr>
<tr>
<td></td>
<td>ICAM-2</td>
<td>LFA-1</td>
<td>leukocytes</td>
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<td></td>
<td>JAM-A</td>
<td>JAM-A</td>
<td>vessel wall</td>
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<td></td>
<td>JAM-C</td>
<td>MAC-1</td>
<td>dendritic cells</td>
</tr>
<tr>
<td>soluble factors</td>
<td>interleukin 1</td>
<td>endothelial cells</td>
<td>* (31)</td>
</tr>
<tr>
<td></td>
<td>factor XII</td>
<td>factor XI</td>
<td>* (33, 41)</td>
</tr>
<tr>
<td></td>
<td>VWF</td>
<td>platelets</td>
<td>* (28, 29)</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
<td>various cell types</td>
<td>* (45, 46)</td>
</tr>
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* studies addressing neurovascular disease; PAF: platelet activating factor; VWF: von Willebrand factor

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dothelial injury compared to platelets expressing functional JAM-A (23).

The diversity and abundance of platelet receptors participating in platelet adhesive interactions make them interesting targets in the context of inflammation.

Platelets, stroke and neuro-vascular inflammation

Stroke is among the leading causes of disability and mortality in the industrialized world (24). The incidence of stroke ranges between 4.2–11.7 per 1000 persons per year (24), caused either by intracerebral bleeding or to a large extent by ischemic stroke (24). The development of symptomatic stenosis caused by atherosclerosis of the carotid arteries or embolism is closely related to vascular inflammation (25). Besides cells of the immune system, platelets are involved in the pathogenesis of ischemic stroke (8).

Platelet adhesion receptors and stroke

One of the experimental settings used to investigate the contribution of platelets to stroke in mice is the tMCAO model, in which transient occlusion of the middle cerebral artery (MCA) is induced using an intraluminal thread (7). Several mechanisms of thrombus formation in the ischemic brain have already been elucidated (7, 8). One important factor is GPIbα (Fig. 1). Blockade of this receptor on platelets via administration of a monoclonal antibody to the second most prevalent platelet receptor GPIbα (GPIbα p0p/B) leads to significantly reduced infarct volumes without affecting platelet count (26). In fact, volume 24h after the onset of tMCAO was reduced to 40% compared to untreated controls (26). Accordingly, animals treated with an antibody against GPIbα showed improved neurological function as assessed by the Bederson score and the grip test (26). Interestingly, no increase in intracerebral hemorrhage could be observed after GPIbα blockade. In contrast to GPIbα, inhibition of GPIIb-IIIa with a monoclonal antibody (JON/A) F(ab)2 one hour before MCAO was unable to improve stroke outcome (26). In contrast, another report showed an effect of GPIIb-IIIa on focal cerebral ischemia in mice deficient for this integrin (27). In this report, differences between GPIIb+/− ApoE−/− and GPIIb−/− ApoE−/− mice that were serving as control group became apparent not only for sites of atherosclerosis such as the aortic arch or the carotid bifurcation, but also after occlusion of the middle cerebral artery or the mouse mesenteric artery (27). Absence of GPIIb (GPIIb−/−) in mice resulted in protection from atherosclerosis in the aortic arch and the carotid artery in histological specimen of the respective vessels (27).

Another important receptor for platelet function is the collagen receptor GPVI (Fig. 1). Blockade of GPVI likewise protects mice from ischemic stroke in the tMCAO model (26). Animals treated with the anti-GPVI antibody JAQ1 (5 days before stroke induction) showed significant improvements regarding stroke volumes whereas neurological function was not significantly improved compared to control animals (26). Intracerebral hemorrhage was neither increased in the GPIIbα nor the GPVI group compared to control animals (26). As animals lacking GPIIbα show reduced infarct volume without augmented intracerebral hemorrhage, it might be possible to inhibit thrombus formation without affecting intracerebral bleeding by a GPIIbα centered approach (7). The main ligand for GPIIbα is VWF especially under conditions of high shear (15). VWF knock-out mice subjected to tMCAO revealed stroke volumes that were reduced to 60% compared with wild type animals, and this was also translated into an improved functional outcome (28). The relevance of VWF and GPIIIa in stroke is underlined by studies in humans. Patients with increased VWF serum levels were at higher risk for developing stroke. Accordingly, in patients showing reduced activity of ADAMTS13, an enzyme important in VWF cleavage, the risk of stroke is markedly increased (29).

Platelets, inflammatory mediators and stroke

In vascular inflammation, the interactions of platelets, leukocytes and endothelial cells are orchestrated by a variety of factors including proteins presented on the platelet surface such as P-selectin or CD40 ligand, interaction with leukocyte receptors such as P-selectin glycoprotein ligand (PSGL-1) or endothelial receptors such as ICAM-1 and CD40 or paracine effectors released by platelets as reviewed elsewhere (30). For neurovascular inflammation of the brain, platelet derived IL1α seems to be of particular importance (31) (Fig. 1). Platelets assemble in close proximity to brain endothelial cells, when mice are subjected to tMCAO (31). Furthermore, primary mouse brain endothelial cells exposed to platelets isolated from wild type mice have a significantly increased expression of surface antigens VCAM-1 and ICAM-1 and secrete relevant amounts of the chemokine CXCL-1, whereas when platelets from IL1α−/− mice were used, no such effect was observed (31). The same effect was observed with conditioned media from WT platelets. Additionally, IL1α induced expression of VCAM-1 and ICAM-1 on brain endothelial cells, which was accompanied by increased transendothelial migration of neutrophils (31).

Platelets at the intersection between the coagulation cascade and stroke

Platelets are placed at the intersection between primary hemostasis and the plasmatic coagulation cascade. Already in 1972, Walsh et al. presented evidence that the activated platelet membrane provides a surface for the assembly and activation of FXII, FXI, the FX activating complex and other components of the coagulation cascade, which was confirmed by following studies; reviewed in Walsh et al. (32). Certain members of the intrinsic coagulation pathway such as factor XII (FXII) critically contribute to the development of acute ischemic brain injury (Fig. 1). FXII knockout mice are protected from stroke after tMCAO (33). This protective effect can be reversed by the reconstitution of FXII knockout mice with FXII (33). Recently, it has been reported that selective inhibition of FXII with recombinant infestin-4 fused to albumin reduced thrombus formation in a mouse model of carotid artery plaque rupture in atherosclerotic ApoE−/− mice (34).
Originally, infestin-4 has been isolated from the mid gut of the blood sucking bug triatoma infestans. Furthermore, significant dislocation of small emboli could be observed in the group treated with FXII inhibitors, whereas this was not the case in the control group. In the same experimental model, this group observed impaired thrombus formation at a more initial state when experimental suppression of FVII activity was performed. The authors’ conclusion was that factor VII is mainly involved in early thrombus formation, while factor XII is necessary for thrombus stabilization (34). Factor XII is closely related to inflammation via the bradykinin kininogen system (Fig. 1). Activated factor XII can result in bradykinin formation and therefore acts pro-inflammatory (35, 36). Besides proinflammatory cytokines and chemokines, platelets can release inorganic polyphosphates, which are negatively charged providing a starting point for the activation of coagulation factor XII and, thus, proinflammatory and procoagulatory reactions (37). The connection between coagulation factor XII and the kallikrein kininogen system has been addressed in several studies regarding the pathogenesis of stroke (36, 38, 39). An interesting finding was that platelets from patients after stroke show an increased reactivity towards thrombin (40). The fact that FXII holds an important role in the pathogenesis of stroke is supported by the observation that a lack of coagulation factor XI, which is activated by activated factor XII, has a protective effect in patients with regards to stroke (41). Furthermore, mice deficient for coagulation factor XI display reduced severity of stroke in the tMCAO model compared to wild type control animals (33). Accordingly, there is data both from basic scientific research and from clinical trials supporting a direct connection of platelets and the intrinsic pathway of coagulation to neurovascular inflammation and stroke.

Although both the relevance of platelet activation and the involvement of the coagulation cascade in stroke development is well established, a potential crosslink in a platelet specific context for example using a platelet Cre/Lox system has not been investigated yet.

Fig. 2 Mechanisms, of how platelets can contribute to disease development of MS or its corresponding mouse model experimentally induced autoimmune encephalomyelitis (EAE). Platelets interact with brain endothelial cells and contribute to leukocyte recruitment via interactions of GPIb/vWF, GPIb/ MAC-1 and P-selectin/PSGL-1. T-cells are recruited by interactions of integrin α4β1/VCAM-1. During EAE, platelets secrete increased amounts of microparticles, which contain mediators modulating inflammation. Whether platelets can reach the subendothelial space during inflammation and contribute to the inflammatory response there, will have to be addressed in future investigations. In the brain parenchyma, T-cells and activated myeloid cells mediate demyelination and axonal damage of neurons. GP: glycoprotein; PSGL-1: P-selectin glycoprotein ligand; vWF: von Willebrand factor.
Data indicate that platelets provide a bounty of mechanisms affecting stroke and its associated processes including inflammation and tissue remodelling. Understanding mechanisms, how platelets contribute to tissue destruction, but also regeneration of damaged neuronal tissue may offer novel urgently needed therapeutic approaches in stroke beyond current and unfortunately limited treatment concepts.

Platelets drive disease development in MS/EAE

Multiple sclerosis (MS) is one of the most common neurological disorders in young adults (42). Especially in Europe and the United States, many people are suffering from this devastating disease (42). The pathogenesis of MS features an interplay between innate and adaptive immunity with a characteristic destruction both of the myelin sheath and the axons causing focal central nervous system damage and neurological deficits (43, 44) (Fig. 2). Increasing evidence suggests a substantial contribution of platelets in this context (9). For instance, clinical symptoms in an animal model of experimental induced autoimmune encephalomyelitis (EAE, one corresponding mouse model of the human disease) correlated with levels of platelet-activating factor (PAF) (45). This finding was consistent with the observation of increasing PAF levels in the CSF of individuals suffering from relapsing MS (46). Furthermore, platelets have been detected in lesions of the central nervous system in both patients with MS and animals with EAE (47, 48) (Fig. 2). This went along with an increase of mRNA for platelet surface antigens GPIb and GPIIb in mice after induction of EAE (47). In report a murine model of Alzheimer’s disease addressing the contribution of platelets to disease development revealed only few platelets migrating into the brain parenchyma (49). Furthermore, histological specimen of humans suffering from Alzheimer’s disease showed only few platelets being directly present in the brain parenchyma (49). However, in humans platelets were detected associated with Alzheimer plaques or vessels close to Alzheimer plaques (49). As EAE/MS and Alzheimer’s disease feature differing pathophysiologicals, future research is required to answer the question of platelets being present in the brain parenchyma in MS/EAE or also other diseases with neurovascular inflammation.

A study primarily investigating the relevance of P-selectin and E-selectin in EAE could localize P-selectin immunostaining to CD41-positive platelets adhering to the vessel wall (50). Furthermore, a proteomic approach clearly demonstrated that in chronic active MS lesions, proteins of the coagulation system as well as proteins of the megakaryocytic lineage were present (51). In control samples from people not affected with MS, these proteins were not detectable suggesting a potential contribution to disease pathogenesis (51). Moreover, platelets represent a circulating pool of inflammatory mediators, for instance non-neuronal serotonin (52). It was shown that platelet-derived serotonin can modulate inflammation by promoting neutrophil recruitment in different experimental settings (53). Indeed, treatment of MS patients with selective serotonin reuptake inhibitors reduced the number of newly formed lesions in patients with relapsing MS (54). Another support for a role of platelets in MS comes from clinical studies. Two studies could show that markers of platelet activation indicated by enhanced P-selectin expression and increased microparticle production are elevated in the blood of patients suffering from MS (55, 56). Interestingly, platelet-derived microparticles were significantly increased in untreated MS patients compared to controls correlating with highest levels during inflammatory periods indicating a potential role for MS pathogenesis (56). Furthermore, association of IgM with platelets was significantly elevated whereas the study could not show differences for protein S, an important protein of the soluble coagulation cascade (55).

Assessing the relevance of platelets for neuroinflammation, experimental depletion of platelets was performed in EAE leading to an ameliorated disease course, particularly in the effector phase of the disease (47). This was paralleled by reduced activation of microglial cells and proinflammatory cytokines (47). Experimental depletion of platelets in mice undergoing EAE also revealed smaller areas of demyelination in the central nervous system (47). Platelets are able to interact both with the endothelium and leukocytes providing a great abundance of potent surface receptors (4, 22, 47). So far, reports from experiments in animals could show that platelet surface receptors GPIbα and GPIIb-IIIa contribute to the pathogenesis of EAE (Fig. 2). Experimental blockade of platelet receptors GPIbα and GPIIb-IIIa, respectively, with blocking Fab showed milder course of EAE (47). Furthermore, blockade of MAC-1 a binding partner of GPIbα on leukocytes with Fab also leads to less severe disease in mice after induction of EAE (47). One immunomodulatory drug for patients with MS is glatiramer acetate (GA). It was shown that GA also has a strong influence on platelet function (57).

When platelets are treated with GA, expression of platelet marker GPIbα-IIIa and P-selectin are significantly decreased in mice and humans (57). Calcium influx into thrombin stimulated platelets becomes diminished when platelets are pretreated with GA before stimulation with thrombin (57). In accordance with an impaired platelet function in vivo, tail bleeding time in mice was prolonged when GA was applied (57). Taken together, platelet research can potentially offer valuable new insights into the pathophysiology of MS and might provide novel therapeutic options for its treatment.

Conclusions

Interactions between platelets, the endothelium and leukocytes initiate and sustain inflammation of larger vessels and the microcirculation of neuronal tissues. This response is mediated by cell-cell interactions and paracrine mediators.

Neurovascular inflammation confers tissue damage in neuronal diseases such as stroke or MS. While the contribution of vascular endothelium and leukocytes to these neuronal diseases has been well recognized and therapeutic approaches such as the inhibition of adhesion receptors
in MS have been introduced into clinical practice, the pathophysiological role of platelets in MS or neurodegenerative disorders or their effect in tissue remodelling beyond mediating thrombosis has only recently moved into the focus of research. Following these novel and innovative concepts and bringing together experts of different fields of research may, therefore, open new therapeutic possibilities to combat these severe diseases.

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Conflict of interest
The authors declare that they have no conflict of interest.

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