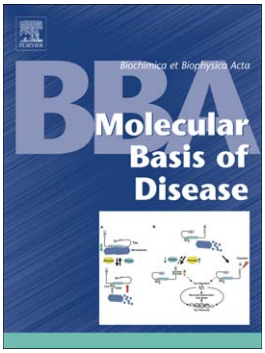

The plasminogen activation system in neuroinflammation

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The plasminogen activation system in neuroinflammation

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HIGLIGHTS

> The PA system is upregulated in inflammatory conditions

> Several components of the neuroinflammatory response are influenced by the PA system

> The PA system promotes a global pro-inflammatory effect

> The PA system acts in neuroinflammation *via* proteolytic and non-proteolytic mechanisms

ABSTRACT

The plasminogen activation (PA) system consists in a group of proteases and protease inhibitors regulating the activation of the zymogen plasminogen into its proteolytically active form, plasmin. Here, we give an update of the current knowledge about the role of the PA system on different aspects of neuroinflammation. These include modification in blood-brain barrier integrity, leukocyte diapedesis, removal of fibrin deposits in nervous tissues, microglial activation and neutrophil functions. Furthermore, we focus on the molecular mechanisms (some of them independent of plasmin generation and even of proteolysis) and target receptors responsible for these effects. The description of these mechanisms of action may help designing new therapeutic strategies targeting the expression, activity and molecular mediators of the PA system in neurological disorders involving neuroinflammatory processes.

INTRODUCTION

The plasminogen activator (PA) system refers to a group proteins involved in the regulation of the activation of the zymogen plasminogen to the active serine-protease plasmin. This includes the two plasminogen activators (tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA)) and a series of inhibitors of their activity, principally plasminogen activator type 1 (PAI-1) and neuroserpin (NS) (Figure 1).

The PA system has two classical functions: the regulation of extracellular matrix (ECM) degradation in most tissues, and the regulation of fibrinolysis in the bloodstream. In the ECM, plasmin activates the pro-forms of matrix metalloproteases to their active form, which are able to degrade ECM components. This function is important for prevention of fibrosis, but also in migration and cell process growth, in which ECM is a physical obstacle. In the bloodstream, plasmin directly cleaves fibrin into fibrin degradation products. This function is important for the prevention of fibrin clot formation responsible for vascular occlusions and is also the basis of thrombolysis (i.v. injection of recombinant tPA), the only approved pharmacological treatment of ischemic stroke.

Neuroinflammation is a common feature to several CNS diseases such as stroke, Alzheimer's disease and multiple sclerosis (MS). A neuroinflammatory response occurs from the acute phase of ischemic stroke and is characterized by the production of inflammatory mediators (cytokines, chemokines and adhesion molecules), overt blood brain barrier leakage and leukocyte entry to the CNS (early neutrophil infiltration, followed by monocytes). Alzheimer's disease is considered a chronic neuroinflammatory disease, in which misfolded and aggregated proteins trigger microglial activation, leading to the release of inflammatory mediators which contribute to disease progression. Neuroinflammation is also a key feature in the pathology of multiple sclerosis: adhesion molecules and inflammatory cytokines lead to

the infiltration of neutrophils, monocytes and lymphocytes through an altered blood-brain barrier, which trigger autoimmune attack against myelin.

In the recent years, the spectrum of actions of the PA system has extended far beyond these classical functions. In particular, the PA system has been reported to influence several cellular and molecular determinants of inflammation, making it a key player in the regulation of neuroinflammation. Because tPA is the active molecule used for thrombolysis in stroke, part of the studies presented here have addressed the effects of the PA system on neuroinflammation in the context of stroke. However, the different actors of the PA system can also display unforeseen effects, some of them independent of the activation of plasminogen, in a more general context of neuroinflammation, with implications in other pathologies, such as multiple sclerosis or Alzheimer's disease. In this review, we will explain how the PA system acts on several aspects of inflammation, such as blood-brain barrier function, leukocyte diapedesis, intraparenchymal fibrinolysis, microglial activation or neutrophil functions. We will focus on the cellular targets and the molecular mechanisms responsible for these effects.

Sources of the different actors of the PA system in the brain

Tissue-type plasminogen activator (tPA)

The presence of a fibrinolytic/plasminogen converting activity in the brain was reported as early as in the 70's [1][2]. Ten years later, this activity was reported to in fact result from two actors, identified as being tPA and uPA[3].

Endothelial cell forming microvessels (<100 µm of diameter) are the main source of tPA in the brain [4][5]. However, virtually all cell types within the central nervous system are potential sources of tPA. The expression of tPA by neurons has been largely documented *in vitro* and *in vivo* [6][7][8][9]. The presence of tPA mRNA and protein/activity in cultured astrocytes is also well documented [7][9][10][11][12] but in a lesser extent *in vivo* [13][14]. Oligodendrocytes had formerly been shown to be devoid of tPA activity *in vitro* [15], but they have recently been shown to display a positive immunostaining for tPA *in vivo* [16].

The ability of microglia to produce tPA remains debated. Indeed, some authors reported the absence of tPA mRNA in microglia [16][7][10] while others detected it [8][17][18][19][20]. Similar controversial results exist regarding tPA protein and activity: for instance, cultured microglia display tPA activity[21], while *in vivo*, no tPA immunostaining is reported in microglia of the hippocampus[22]. A possible explanation for these discrepancies is that, as discussed later in this review, tPA expression, although low in basal conditions, could be induced in microglia during inflammatory processes.

Other sources of tPA in the central nervous system are perivascular mast cells [23], pericytes [24][25], infiltrating leukocytes [26]and blood-derived tPA. This highlights the role for tPA at the blood-brain interface. Indeed, tPA can cross the blood-brain barrier towards the injured brain and aggravate the extent of neuronal loss[27][28]. Low density lipoprotein receptor-related protein (LRP), a transmembrane protein involved in endocytosis, has been implicated in the extravasation of tPA across the blood-brain barrier. *In vitro* data suggest that this

passage occurs via LRP-dependent transcytosis under normal conditions, or via a LRP-independent pathway under ischemic-like conditions[29]. These data underline the fact that tPA present in the brain can originate from synthesis by brain cells as well as from entry from the circulation.

Urokinase plasminogen activator (uPA)

uPA expression in the healthy brain is low, mainly restricted to astrocytes and to a few populations of neurons[30][31][32]. Its expression in the brain is however enhanced in pathological conditions such as epilepsy [32] or inflammatory lesions in multiple sclerosis[33]. The proteolytic activity of uPA is regulated by its binding to the cell surface receptor uPA receptor (uPAR). uPAR is a glycosyl phosphatidylinositol (GPI)-anchored protein which binds uPA and its pro-form (pro-uPA).

Plasminogen

As discussed throughout this review, while some functions of tPA in the brain occur by a direct effect on target effectors, some others require the activation of plasminogen into plasmin. Therefore, the issue of the origin of plasminogen in the healthy and injured brain appears critical and has been a subject of debate in the last years, particularly regarding data obtained from plasminogen knock out mice. The question whether plasminogen is synthesised by brain cells and/or comes from the circulation by crossing the BBB is still open. Also, the possibility has been raised that plasminogen synthesis could be silenced in basal conditions and up-regulated following neuronal activity or brain injury.

The main source of plasminogen in the body is the liver, from where it is released into the circulation with an estimated concentration on a micromolar range[34]. In the brain, plasminogen is secreted by neuroendocrine tissues[35][8] and expressed in the cortex,

hippocampus and cerebellum [36]. In addition, plasminogen mRNA and protein were detected in the mouse in neurons -but not in glial cells- of the hippocampus,[8][37][38]. *In vitro*, cortical neurons subjected to nerve growth factor (NGF) application reveal an increased expression of the mRNA encoding for plasminogen[39]. Overall, while plasmin activity in the normal rat brain is low, it is possibly increased during axonal growth[40] , after brain injury[41], or throughout regenerative events such as spine pruning[42].

Consequently, plasminogen/plasmin activity within the brain parenchyma may be involved in processes related to brain development, learning and memory, brain diseases and brain recovery, as revealed by studies using plasminogen knock out animals. However, only a relatively low number of studies describe brain-related phenotypes for plasminogen knock out mice in health and disease:

Most of the brain-related phenotypes for plasminogen knock out mice have been described during experimentally-induced brain lesions. For instance, Plasminogen knock out are resistant to excitotoxic neurodegeneration induced by kainate infusion[43]. In these conditions, plasminogen was postulated to act by the proteolytic degradation of the extracellular matrix[8][44], thus promoting anoikis, a mechanism of cell death due to loss of anchorage.

Serine proteases inhibitors: PAI-1 and Neuroserpin

The two main tPA inhibitors in the brain are plasminogen inhibitor-1 (PAI-1) and neuroserpin (NS). PAI-1 is an irreversible inhibitor of tPA and uPA activity. PAI-1 is synthesized mainly by endothelial cells in the vasculature but also by astrocytes in the brain[9]. NS is a transient inhibitor of tPA and uPA activity. It is expressed principally in the neurons of the central and peripheral nervous systems[45]. NS forms short-lived and unstable complex with plasminogen activators leading to the cleavage of NS and the liberation of the active enzyme *in vitro*[46]. Thus neuroserpin may be considered as a “plasminogen activator buffer”. When

cleared, the NS-tPA complexes removed from the synaptic space by astrocyte-derived LRP receptors are not necessarily degraded[47]. Further studies should be investigated to understand the fate of NS-tPA complexes cleared from the synaptic space. A possible role of NS in the recycling of tPA may be further investigated. In fact, it seems that even if NS has a lower inhibitory constant than PAI-1, it plays a central role in the bioavailability of tPA in the brain by buffering its presence and its activity.

NS expression can be regulated in pathological conditions, such as in the brain of Alzheimer's disease, possibly in relation to thyroid hormone response [48].

To summarize the information available concerning the expression of the actors of the PA system in the brain, it can be stated that the PA system is upregulated in the brain as a response to inflammation. Accordingly, the actors of the PA system may be used as biomarkers in neuroinflammatory conditions, with specific profiles depending on the pathology. For instance, tPA concentration in the CSF positively correlates with amyloid-β levels [49]. Discrete polymorphisms in the tPA and PAI-1 genes may also represent risk factors for MS, which raises hopes for genetic diagnosis/prognosis [50].

Roles of the PA system in neuroinflammation

tPA increases Blood-Brain Barrier (BBB) permeability and leukocyte diapedesis

tPA induces a loss in BBB function, characterized by an increase in vascular permeability in the early stages of BBB opening [51], enhanced trans- and para-cellular transport [52], increased monocyte diapedesis [53]and -as an ultimate point to BBB breakdown- hemorrhage, a well known complication of tPA-induced thrombolysis in ischemic stroke patients.

LRPs play a vital role in mediating the effects of tPA on BBB [54] (**Figure 2**). Neurovascular permeability is altered when tPA cleaves the endothelial LRP at its substrate binding

ectodomain (a process termed as shedding) which triggers signaling cascades [51]. tPA-induced LRP1 signaling cascade channelizes NF-κB activation which promotes synthesis of MMP-3 and MMP-9 which in turn initiate matrix protein degradation and BBB leakage [55] [56]. In addition, a similar mechanism exists at astrocyte endfeet: shedding of LRP activates NF-κB and Akt mediated signalling [57][58], leading to the expression of MMP-9, which promotes the detachment of the end-feet projections of astrocytes [59] [60]. Because astrocytes have been shown to internalize tPA via LRP1[61], further studies may determine as to whether the above described LRP/tPA mediated signaling cascade in astrocytes depends on the endocytosis of tPA or not.

Platelet-derived growth factor receptor-α (PDGFR-α) also mediates some of the effects of tPA on BBB (**Figure 2).** tPA converts PDGF-CC to active PDGF-C, able to binds with PDGFR-α on astrocytes. This finally induces an increase in BBB permeability by a yet unknown mechanism[62]. The conversion of PDGF-CC to active PDGF-C is abolished in LRP1-/- animals[62], suggesting that the activation of PDGF-CC may be facilitated by the binding of tPA to LRP1 on astrocytes.

N-methyl-D-aspartate (NMDA) glutamate receptors have also been implicated in tPA-induced loss of BBB function. NMDA receptors are expressed on endothelial cells [63] and tPA enhances NMDA receptor function in neurons [64] . In fact, in endothelial cells, blocking NMDA receptors abolishes tPA-induced activation of the Erk1/2 pathway[63]. Interestingly, leukocyte transmigration through endothelial cells is reduced by antagonizing NMDA receptors, by blocking tPA activity, or by blocking the interaction between tPA and NMDA receptors[63]. These data suggest that tPA enhances NMDA receptor-induced Erk pathway in endothelial cells, leading to enhanced leukocyte diapedesis.

The mechanisms by which endothelial NMDA receptors drive an increase in BBB permeability include toxicity (mediated by ROS and peroxynitrite production), and disruption

of tight junctions (mediated by the inhibition of the expression and/or modification of the phosphorylation of occludin) [65][66][67].

tPA can also induce the expression of adhesion molecules on the neuroendothelium, thus facilitating leukocyte infiltration. In cultured brain-derived endothelial cells and in the EAE model of MS, the administration of tPA induces the expression of intercellular adhesion molecule 1 (ICAM-1) by endothelial cells [68]. Altogether, these studies agree for a deleterious effect of tPA on BBB function through complementary mechanisms targeting endothelial cells and astrocytes, and dependent on the proteolytic activity of tPA.

tPA promotes intraparenchymal fibrinolysis

Fibrin is not found in the CNS under physiological conditions, but increase in BBB permeability in inflammatory conditions can lead to entry of fibrin and/or fibrinogen from the blood [69] . The resulting fibrin deposits promote perivascular microglia clustering in MS-like lesions[70], leading to axonal damage [71]. In addition to contributing to axonal damage, fibrin can also limit the capacities of axonal regeneration [71]. tPA, by promoting a fibrinolytic activity within the inflamed brain parenchyma, helps removing fibrin deposits and thus protects against axonal damage in inflammatory conditions (**Figure 3**): in neuroinflammatory conditions such as multiple sclerosis (MS), efficient fibrin removal is impaired, and this is associated with a decreased expression of tPA and an increased expression of PAI-1 [33][72] . Accordingly, in experimental autoimmune encephalitis (EAE, a model of MS), tPA knock out mice show more severe symptoms and impaired recovery [73][74] whereas PAI-1 knock out mice show a delayed onset and less severe symptoms [75] . Moreover, PAI-1 antagonists reduce the severity of symptoms in the same model and this effect is additive to immunomodulatory drugs such as fingolimod [76].

Together, these findings suggest that tPA, by proteolysis-dependent removal of fibrin deposits within the CNS, limits axonal damage in inflammatory conditions.

Plasminogen activators promote microglial response

tPA promotes microglial activation independently of its proteolytic activity, via a "cytokine" effect [77]. This effect is mediated by binding of tPA to the membrane protein annexin II [77] (**Figure 4**). Annexin II has been suggested to co-operate with Galectin-1, for microglial activation by tPA [78], and interestingly, Galectin-1 has been previously described as a tPA receptor in non-CNS cells[79][80]. Aforesaid receptors converge to trigger Akt, Erk 1/2 and Jnk pathways, responsible for microglial activation and inflammatory responses [78].

tPA effects on microglial activation can also be triggered by proteolytic activity. For instance, in cerebral ischemia, high degree of inflammation and excessive microglial activation are a result of enhanced proteolytic tPA activity, an effect which is suppressed by neuroserpin [81]. This highlights the role of proteolytic activity of tPA in microglial activation, but also the role of the brain specific serine protease inhibitor NS in limiting microglia-dependent inflammatory processes. Also in experimental stroke, microglial recruitment was shown to be enhanced by tPA via the upregulation of the chemokine (C-C motif) ligand 3 (CCL3) [82]. The proteolytic effects of tPA on microglial activation and/or recruitment may depend on plasminogen conversion to plasmin, because plasmin also induces microglial activation via its proteolytic activity[83].

An autocrine amplification loop may exist for tPA action on microglial activation: tPA released from neurons triggers microglial activation and, in return, activated microglia release more tPA, triggering inflammation and neuronal toxicity [84]. Importantly, the effect of tPA on microglial activation is accompanied by a polarization to M1 (pro-inflammatory) phenotype [85].

uPA induces *in vitro* microglia migration via its proteolytic activity[86]. Indeed, in experimental spinal cord ischemia, MMP-9 facilitates microglia invasion and migration[87].

uPA receptor (uPAR) may also play a role in microglial response during inflammation. Cell surface expression of uPAR has been considered as a marker of microglial activation [88]. Inflammatory responses in Alzheimer's disease are associated with microglial activation via initiation of uPAR surface expression in microglia and may be affiliated with oxidative stress [89]. Interestingly, while uPAR expression is upregulated in both acute and chronic inflammation, uPA overexpression is restricted to acute conditions[90]. This suggests that uPAR has effects on microglia independently of uPA proteolytic action. Further studies may explain the dissociation between uPA and uPAR effects on microglia in chronic *vs.* acute neuroinflammation.

Overall, these studies converge toward a pro-inflammatory effect of plasminogen activators on microglial response, with proteolytic as well as non-proteolytic effects.

tPA, uPA and uPAR enhance neutrophil function

Neutrophils are a target of the PA system during inflammation. In particular, tPA and uPA have been reported to increase neutrophil activation, degranulation and transmigration.

tPA was shown to promote neutrophil transmigration via the activity of MMP-9 (**Figure 5a**). This effect is due to tPA induced neutrophil degranulation, leading to the release of MMP-9, MMP-8, elastase and myeloperoxidase[91][92]. In addition, tPA, *via* the generation of plasmin, can also activate MMP-9 from its pro-form, thus contributing to MMP-9 mediated action on neutrophil transmigration. During recombinant tPA(actilyse®)-induced thrombolysis in ischemic stroke, given its capacity to activate MMP-9(see Blood Brain Barrier paragraph in this review), tPA is a key operator in inducing haemorrhage, which outlies as a complication of treatment[93]Nevertheless, tPA-induced release and/or activation

of MMP-9 also induces more moderate and reversible loss of BBB function, without overt BBB breakdown, but leading to increased permeability to macromolecules [94] and neutrophil transmigration[94].

tPA-induced degranulation of neutrophils is mediated through the activation of PI3K/Akt and Erk1/2 pathways[92]. Although the receptor on neutrophils responsible for this effect has not been identified yet, still neutrophil responses elicited by tPA were shown to implicate its both proteolytic and nonproteolytic properties[94], in addition to involve the kringle domains of tPA[94]. As an attempt to conciliate reports of proteolytic vs. non proteolytic effects of tPA on leukocyte transmigration, the following model could be proposed: On one hand, tPA would employ its non-proteolytic effects (possibly mediated via binding of Kringle domains to a still undefined receptor on neutrophils) leading to leukocyte degranulation and release of MMP-9; on the other hand, tPA would engage its proteolytic effects (via the activation of plasminogen to plasmin) leading to activation of pro-MMP-9 to active MMP-9 (**Figure 5a**).

The fact that neutrophil infiltration is reduced in tPA-/- animals [94] raises the question of the endogenous source of tPA responsible for this effect. In fact, both endothelial and leukocyte tPA contribute to neutrophil transmigration[94], as shown by cell transfer experiments. Although this mechanism was described in a model of scrotal muscle ischemia and therefore not in the CNS, these data bring the exciting hypothesis according to which tPA produced by neutrophil would promote neutrophil transmigration by an autocrine action. This hypothesis needs to be addressed in further studies using models of CNS injuries.

Mast cells could sustain an additional effect of tPA, in addition to the above-described processes, which could amplify neutrophil transmigration. tPA could activate mast cells and induce them to produce lipid mediators such as leukotrienes, which could amplify inflammation in general, and in particular neutrophil transmigration[94]. Interestingly, mast

cells are also a source of tPA [95] and could thus participate in the cellular cross-talk leading to neutrophil transmigration (**Figure 5a**).

uPA acts in parallel to tPA by promoting neutrophil activation (**Figure 5b**). uPA has been shown to increase the expression of pro-inflammatory cytokines (IL-1β, MIP-2 and TNF) by LPS-activated neutrophils[96]. This effect is mediated through the activation of Jnk pathway, and nuclear translocation of NF-KB[96]. To activate this transduction pathway, uPA acts in a non-proteolytic fashion by binding the α-V-β-3 integrin via its kringle domain[97]. A study in muscle ischemia (and thus not necessarily transposable to situations of neuroinflammation) reported that another integrin, Mac1/CD11b (α-M-β-2 integrin), may serve as a receptor for uPA in the process of neutrophil recruitment[98].

Strikingly, PAI-1 was shown to induce the same effects as uPA in LPS-activated neutrophils, *i.e.* activation of Jnk pathway, nuclear translocation of NF-κB and enhanced production of pro-inflammatory cytokines[99]. Indeed, uPA and PAI-1 were shown to have additive effects on neutrophil activation[99]. Of note, the effects of PAI-1 on neutrophil activation were independent of its protease inhibitory action[99].

In summary, tPA and uPA exert individual, though complementary actions to increase neutrophil recruitment: tPA, via proteolytic and non-proteolytic actions, promotes neutrophil transmigration, while uPA, via non-proteolytic actions, promotes neutrophil activation.

Interestingly, the above reported effects of uPA are displayed independently of uPAR. On top of this, uPAR was also reported to play effects on neutrophils independently of the binding of uPA. uPAR can cluster, at least in part independently of uPA, to induce intracellular Ca2+ raise which leads to enhanced surface expression of CD11b/CD18, contributing to an adherent phenotype of neutrophils[100]. Since uPAR binding to CD11b/CD18 facilitates neutrophil transmigration[101], a two-step process can be proposed: first, uPAR clustering on

neutrophils increases CD11b/CD18 expression, leading to neutrophil adhesion; second, uPAR binding to CD11b/CD18 leads to transmigration.

In addition to these effects on neutrophil transmigration, uPAR expression on neutrophil constitutes a non-conventional "don't eat me" signal for macrophages. This signal inhibits efferocytosis[102], a process leading to the engulfment of neutrophils by macrophages during the clearance of the inflammatory process. Noteworthy, uPAR also exists as a soluble form shed from activated neutrophils, which was suggested to promote chemoattractant properties for monocytes[103]. Together, these data indicate that uPAR, either as its membrane form or as its soluble form, regulates the communication between neutrophils and monocytes/macrophages.

Altogether, these data indicate that distinct actors of the PA system (here, tPA, uPA and uPAR), can act together to promote the distinct aspects of neutrophil reaction: activation, adhesion, transmigration and efferocytosis. Noteworthy, these different actions coincide towards a global pro-inflammatory effect.

Conclusions

The plasminogen activator (PA) system, originally described for its role in regulating fibirinolyis in the bloodstream, has emerged as a central actor in neuroinflammatory processes. The different actors of the PA system are up-regulated in response to brain insults and regulate several aspects of inflammation, such as loss of BBB permeability, leukocyte diapedesis, intraparenchymal fibrinolysis, microglial response and neutrophil function. These actions of the PA system are directed towards a global pro-inflammatory effect, except for intraparenchymal fibrinolysis, which limits microglial clustering. Because activated microglia releases tPA, this process could be considered as negative feedback mechanism in neuroinflammation. Some of the cellular actions of the PA system have been reported in the

context of inflammation in general, and not specifically for neuroinflammation. Future investigations in appropriate animal models may thus apply this knowledge to specific neuroinflammatory conditions. Also, some studies suggest that the PA system acts differently on acute and chronic inflammation, which should be further investigated. Finally, study of the fine mechanisms of action of the PA system in neuroinflammation reveals that previously unsuspected protease-independent, "cytokine" effects, act in addition to classical protease-dependent effects. Overall, further investigations may bring new treatment strategies targeting the expression, activity and molecular mediators of the PA system in neuroinflammation.

FIGURES :

Figure 1: The plasminogen activator system. Tissue-type- and Urokinase- plasminogen activators (tPA and uPA) activate the inactive zymogen plasminogen (Plg) into active plasmin (Plm). The activity of tPA and uPA is inhibited by serine proteases inhibitors (serpins), mainly Plasminogen activator inhibitor -1 (PAI-1) and neuroserpin (NS).

Figure 2: tPA effects on the Blood brain barrier. tPA acts on LRP receptors on endothelial cells and astrocytes. In both cases, this effect is mediated through its proteolytic action (« shedding » of LRP receptors), leading to the recruitment of NFκB and the production of MMP9 by target cells. MMP9 then plays its action of BBB, enhancing its permeability and leukocytes diapedesis. In endothelial cells, NMDA receptors may also in part mediate tPA effects. Alternatively, the proteolytic activation of PDGF-CC in active PDGF-C by tPA can also contribute to astrocyte endfeet detachment by activating astrocytic PDGFR-α. The activation of PDGF-CC is facilitated by the binding of tPA to LRP receptors on astrocytes.

Figure 3: tPA effects of intraparenchymal fibrinolysis and the link with microglia. (a) In conditions of neuroinflammation, fibrinogen can enter the CNS parenchyma through an impaired BBB. Once in the tissue, fibrin(ogen) can interact with the CD11b/CD18 integrin, inducing microglia activation and clustering. **(b)** tPA can degrade fibrin into fibrin degradation products, a process termed as intraparenchymal fibrinolysis, which limits fibrin-induced microglia activation and clustering. Because activated microglia releases tPA, this process could act as a negative feedback control on microglial function.

Figure 4: Cytokine-like effects of tPA on microglia. tPA induces non-proteolytic, « cytokine-like » effects on microglia, mediated by Annexin II, possibly via a co-operation with Galectin 1. The interaction of tPA with these membrane targets leads to the activation of microglia *via* Akt, Erk1/2 and Jnk pathways, leading to enhanced production of pro-

inflammatory cytokines. In addition, activated microglia can release tPA, which contributes to an autocrine amplification loop.

Figure 5: Effects of tPA and uPA on neutrophils. (a) tPA enhances the degranulation of neutrophils by a non-proteolytic activation of a yet-unknown receptor activating PI3K and Akt pathways, and leading to the release of MMP9. Alternatively, via its proteolytic activity, tPA can activate pro-MMP9 to active MMP9. MMP9 then increases blood brain barrier permeability and leukocyte diapedesis. In parallel, tPA orchestrates a cross talk between neutrophils and mast cells: tPA can activate mast cells, leading to release of lipid mediators which aggravates the effects on the BBB. Mast cells, in return, can produce an additional pool of tPA. In addition to its recombinant form injected in the context of thrombolysis, tPA originates from endothelial cells, mast cells, and possibly neutrophils to induce autocrine actions. **(b)** uPA induces neutrophil activation, leading to the release of pro-inflammatory cytokines. This action is dependent on the Kringle domain of uPA, and occurs through the interaction with αVβ3 integrin, activating Akt and Jnk pathways and subsequent recruitment of NFκB.

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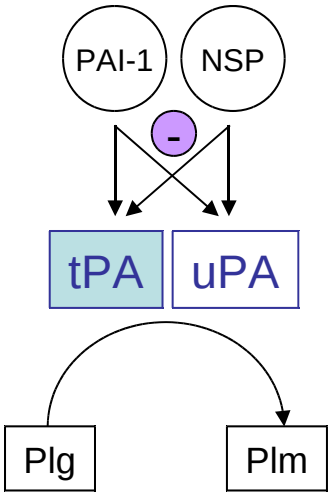


Figure 1

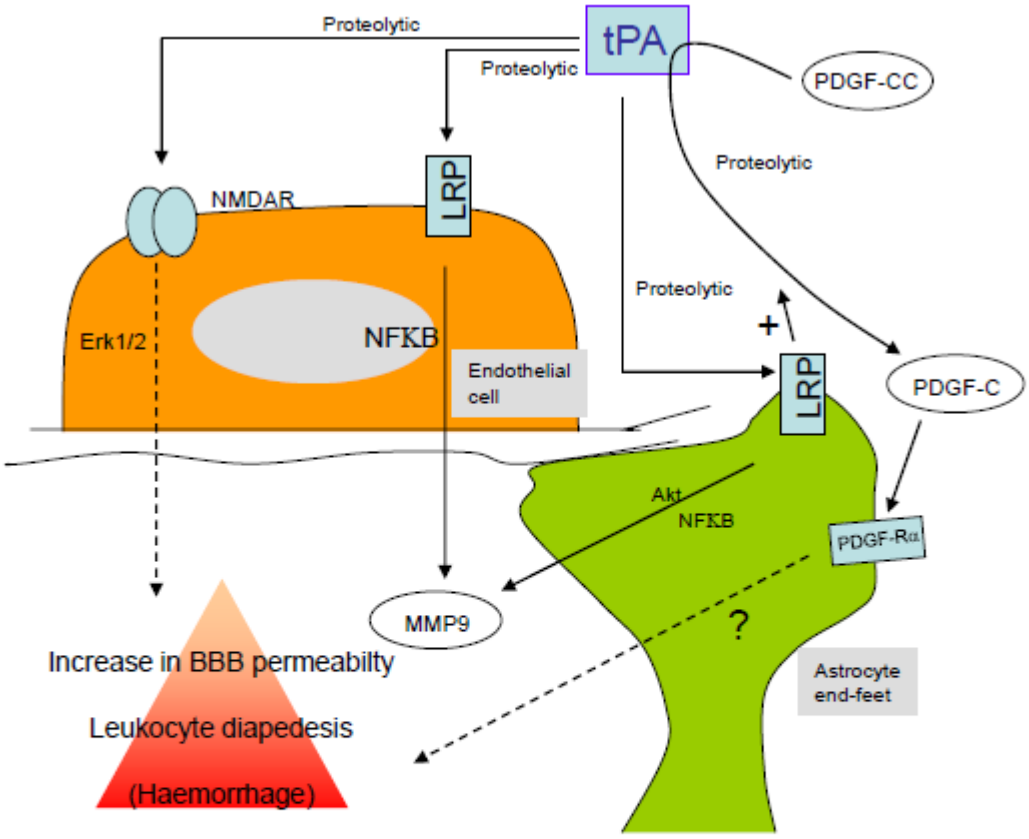


Figure 2

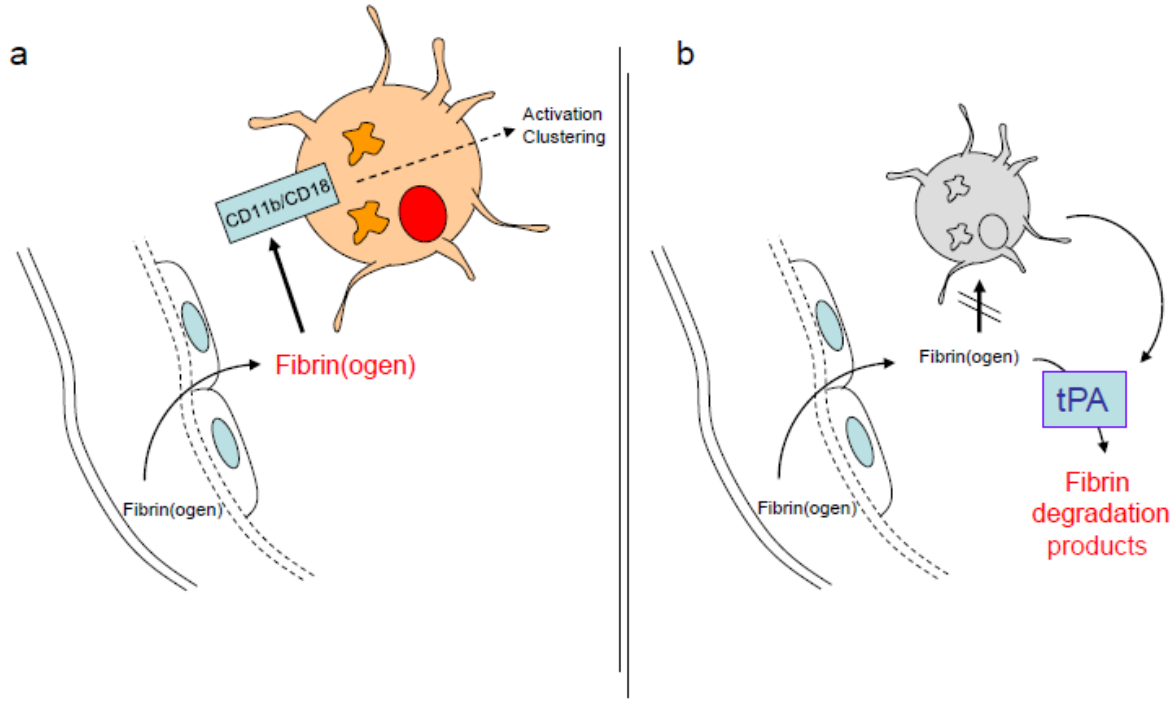
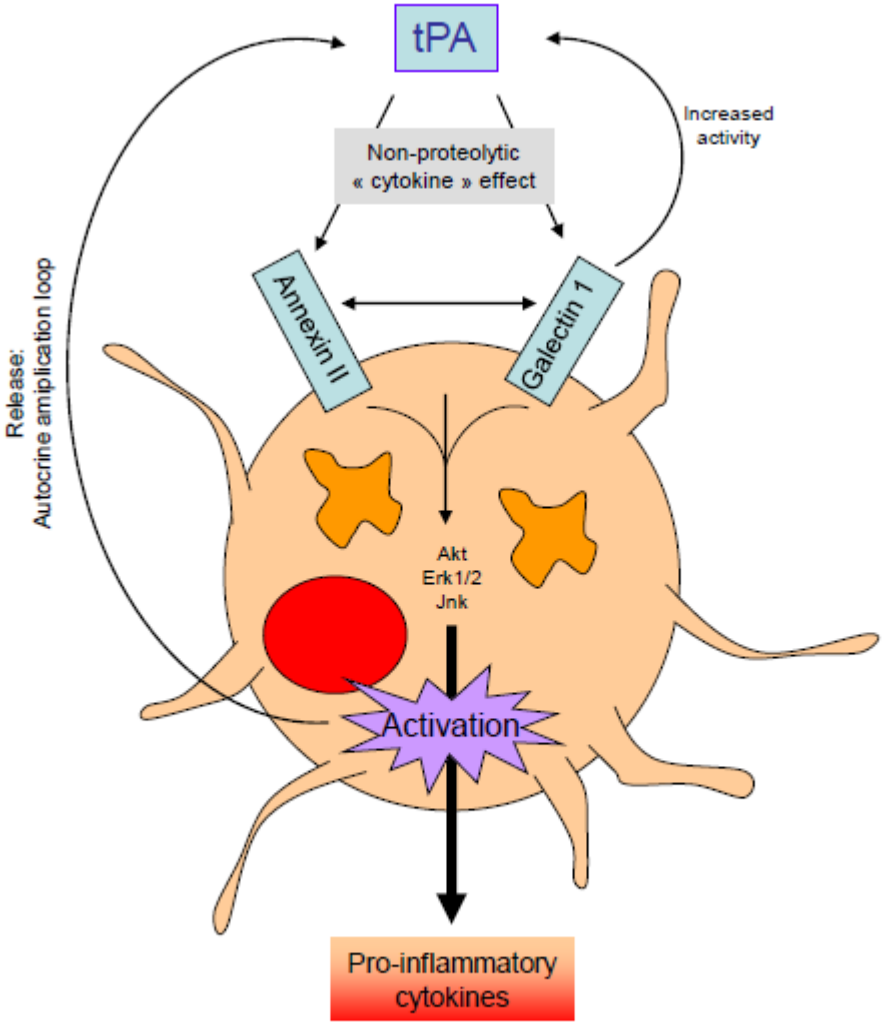


Figure 3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Non-proteolytic
« cytokine » effect
Increased
activity
Release:
Autocrine amplication loop
Annexin II
Galectin 1
Akt
Erk1/2
Jnk
Activation
Pro-inflammatory
cytokines

Figure 4

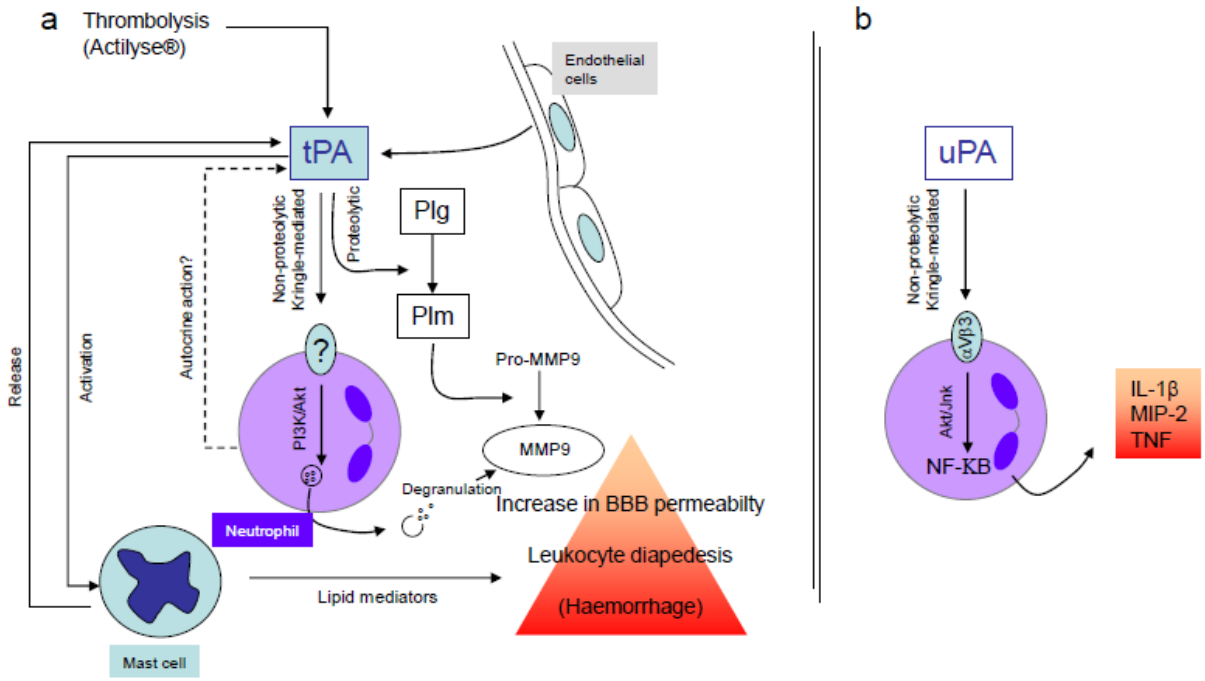

Figure 5