Summary. We all know about classical fibrinolysis, how plasminogen activation by either tissue-type plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA) promotes fibrin breakdown, and how this process was harnessed for the therapeutic removal of blood clots. While this is still perfectly true and still applicable to thromboembolic conditions today, another dimension to this system came to light over two decades ago that implicated the plasminogen activating system in a context far removed from the dissolution of blood clots. This unsuspected area related to brain biology where t-PA was linked to a plethora of activities in the CNS, some of which do not necessarily require plasmin generation. Indeed, t-PA either directly or via plasmin, has been shown to not only have key roles in modulating astrocytes, neurons, microglia, and pericytes, but also to have profound effects in a number of CNS conditions, including ischaemic stroke, severe traumatic brain injury and also in neurodegenerative disorders. While compelling insights have been obtained from various animal models, the clinical relevance of aberrant expression of these components in the CNS, although strongly implied, are only just emerging. This review will cover these areas and will also discuss how the use of thrombolytic agents and anti-fibrinolytic drugs may potentially have impacts outside of their clinical intention, particularly in the CNS.

Keywords: blood brain barrier; fibrinolysis; plasminogen; plasminogen activators; stroke; traumatic brain injury.

Introduction

The concept of a blood clotting system emerged over a century ago when the French physiologist, Albert Dastre reported on the time-dependent spontaneous loss of fibrin in clotted blood and coined the term ‘fibrinolysis’ to describe this observation [1]. Having identified this as a blood-related phenomenon led subsequent scientists to explore how this fibrin dissolving system worked, and ultimately how it could be harnessed therapeutically. Blood was the focal point for fibrinolysis and intensive efforts were undertaken in the USA and Europe at the turn of the last century to identify the active molecules in this compartment. The next 50 or so years resulted in impressive insights, particularly given the limited technology at hand during this period. Huge advances were made in the 1930s when an endogenous fibrinolytic activity was first discovered in vampire bat saliva in 1932 [2], and then in haemolytic streptococci in 1933 [3]. Indeed, further investigation into the fibrinolytic moiety in the latter (subsequently referred to as streptokinase) provided the foundation to piece together most of the major components of the fibrinolytic system as we know it today.

The motivation to understand the process of blood clot dissolution necessarily directed attention to the blood compartment. As a result there was scant interest in exploring any possible role of the fibrinolytic system outside of the bloodstream. Of course, there was no desire to look sideways as there was so much potential in understanding and harnessing this process of fibrinolysis for the treatment of thromboembolic conditions. Nonetheless, as soon as assay systems became developed to allow scientists to measure levels of the numerous blood-derived components of the fibrinolytic system in various compartments, it became clear that blood was not the only location housing these molecules. A number of seminal papers from MacFarlane in 1946 [4] and Astrup in 1947 [5] and in the early 1950s [6] reported marked expression of t-PA and anti-plasmin in a variety of human tissues, as well as in seminal fluid [7] and human milk [8]. While these findings could have been viewed as sound evidence that the fibrinolytic system might indeed have a role outside of blood, most commentary in this period still associated this tissue/fluid-derived fibrinolytic activity as being essential to remove fibrin deposits in small vessels and tubular structures within these various tissues. For this reason, the vast majority of scientists actively engaged in fibrinolysis still remained focused on the initial linkage of this system with blood clot and fibrin removal, and to harness this system for therapeutic thrombolysis.

Despite the slowly accumulating knowledge that the fibrinolytic system existed outside the bloodstream, it was
not until the advent of gene knock-out technology before it became apparent that fibrinolysis extended beyond fibrin removal. The arrival of t-PA deficient mice in 1994 [9] sent shock waves through the scientific community, not because of an unexpected phenotype revealed in this seminal publication, but rather because the expected phenotype (i.e. rampant thrombosis) was not seen. Even the simultaneous removal of both t-PA and u-PA did not result in a pro-thrombotic phenotype as was widely anticipated. Perhaps there was another plasminogen activator compensating for the absence of t-PA and u-PA in these genetically modified mice? However, severe thrombosis was not seen in plasminogen deficient mice either [10,11]. This is not to suggest that there were no effects at all, indeed plasminogen deficient mice did show increased fibrin deposition in various organs and displayed a variety of other ailments (conjunctivitis, anal prolapse etc.) and had significant, indeed profound delays in wound healing [12]. However, given the preceding history that blood clot removal revolved entirely around plasmin generation, the degree of perturbation in haemostasis seen in these mice was certainly milder than anticipated. Human plasminogen deficiency was first reported in 1997 [13] and was associated with the development of ligneous conjunctivitis while subsequent studies also found no increase in thrombosis in patients with plasminogen deficiency [14].

That fibrinolysis seemed to be at least partially maintained in plasminogen deficient mice implied that a plasminogen activation-independent fibrinolytic mechanism existed to maintain haemostasis. Indeed, an alternate pathway of fibrinolysis had already been reported by Plow and Edgington in 1975 in a process involving leucocyte-derived proteases [15] and leucocyte-derived elastase was subsequently identified as a likely contender [16]. Later reports using plg<sup>−/−</sup> mice also identified polymorphonuclear (PMN) leucocyte-derived elastase as well as cathepsin G as the compensating fibrinolytic proteases in these mice [17] together with an increase in PMN phagocytic activity [18].

Fibrinolysis and the brain

Experiments conducted in the 1940s had indicated increases in blood fibrinolytic activity following administration of adrenaline [19] or following electroconvulsive therapy [20], situations that were associated at the time with stimulation of the sympathetic nervous system. The first demonstration of fibrinolytic activity in human brain tissue was reported by Frantl and Fitzpatrick, an Australian group, in 1950 [21]. Other reports further indicated strong fibrinolytic activity in cultured gloma cells that coincided with mitotic activity [22]. Histochemical studies on human brain sections revealed fibrinolytic activity in regions that were not necessarily associated with blood vessels suggesting a non-vascular source for the fibrinolytic proteases in the brain [23]. Increased fibrinolytic activity was also detected in brain tumours and also in cerebrospinal fluid (CSF) [24].

More direct evidence that these fibrinolytic components were actually synthesised and expressed in neuronal cells was published almost simultaneously by two groups. In the early 1980s, Nic Seed’s laboratory in Colorado reported that both t-PA and plasminogen was present in post-natal mouse cerebellum [25,26]. At about the same time, a ‘PA’ activity was detected in the mouse brain by Ruth Miskin’s laboratory in Israel [27] where it was stated:

‘Our findings suggest that in the brain PA is produced by neurons and by epithelial cells, and that it may have additional functions to that of thrombolysis both in the developing and the mature brain’.

Ruth Miskin’s group further investigated the intracellular localisation of this PA using homogenates of bovine brain cortex and observed an enrichment in intact synaptosomes, thereby predicting a role in the functioning of nerve terminals [28].

While these studies reported on the steady-state levels of t-PA in the brain, evidence soon appeared to reveal that t-PA gene expression was actually inducible in this compartment. A publication by Qian et al. in 1993 identified t-PA as one of 5 immediate-early genes induced in the brain following either convulsive seizure or high frequency stimulation [29]. This led to speculation that t-PA played a role in ‘structural changes that accompany activity-dependent plasticity’ [29]. A series of transgenic mice developed during this period further intensified the expectation of a key role for t-PA in the CNS. Transgenic mice expressing the first 4 kb of the mouse t-PA gene promoter fused to the Lac-Z reporter gene revealed striking Lac-Z expression in distinct regions of the mouse CNS during development [30]. A similar, although not identical temporal expression pattern in the CNS was reported in neonatal transgenic mice and rats expressing 3.0 kilobase (kb) of the human t-PA gene promoter, also fused to the Lac-Z reporter gene [31]. Subsequent evaluation of the expression pattern generated in adult transgenic mice harbouring 9.5 kb of the human t-PA gene promoter also demonstrated marked yet selective expression in various regions of the brain, particularly in the hippocampus [32]. The consistent expression of t-PA seen in the hippocampal regions of the CNS further strengthened the case that t-PA had a functional role in processes associated with learning and memory. Other transgenic lines expressing the t-PA promoter reporter constructs also identified t-PA expression in sympathetic nerve endings [33] and it was even suggested that plasma levels of t-PA could be derived, in part, from sympathetic nerves.

The availability of various genetically deficient mice finally provided the proof that the plasminogen activating system indeed had an important functional role in the brain. t-PA deficient mice were reported to have reduced...
long term potentiation (LTP), which is the strengthening of synapses due to recent activity. By inference, this was suggestive of a role in memory formation [34] and a series of papers published in later years gave further support for an important role for t-PA in memory formation [35,36]. The laboratory of Jean-Dominique Vassalli in Geneva generated a transgenic mouse line that selectively overexpressed mouse t-PA in neurons (transgene driven by the Thy1.2 gene promoter; ‘T4 mice’). Mice neuronally overexpressing t-PA displayed enhanced spatial (hippocampal-dependent) learning tasks and enhanced LTP [37]. This data also indicated that there was no ‘ceiling effect’ for t-PA under normal conditions, implying that further increases in t-PA expression in the CNS could even be considered beneficial, particularly in conditions associated with cognitive decline.

While transient increases in t-PA may be beneficial at least in the context of LTP, prolonged overexpression of t-PA in the CNS, however, appears to be unfavourable. For example, in genetic mouse models associated with ataxia, i.e. nervous mice [38,39], Lurcher mice [40] and SCA-1 mice [41], elevated cerebellar t-PA levels were associated with pathology. Moreover, the T4 transgenic mice (referred to above) were later reported to display increased Purkinje cell damage and altered gait [42]. Hence, prolonged increases in t-PA expression in the CNS is not well tolerated.

While the association of t-PA with memory formation and LTP had become apparent, another remarkable observation was published in the mid 1990s from Sid Strickland’s laboratory in the USA where it was revealed that t-PA deficient mice were resistant to glutamate-induced neuronal injury [43]. Put more simply, neuronal cell death initiated by excitatory glutamate analogues (i.e. kainic acid, N-methyl-D-aspartate; NMDA), that act via binding to (ionotropic) kainic acid or NMDA surface receptors, respectively, did not occur in the absence of t-PA. Hence t-PA was a critical component in the implementation of glutamate-induced neuronal death. This seminal finding temporarily overshadowed the earlier work showing the effect of t-PA on LTP, mostly because of the potential clinical ramifications. Indeed, this particular publication in Nature appeared in the same year that t-PA was approved for use in patients with acute ischaemic stroke [44]. Hence, on one hand the story was being propagated that t-PA was potentially neurotoxic, yet on the other it had just been considered as a safe treatment (within a limit time window) for patients with ischaemic stroke, a condition that was already known to be associated with excitotoxicity (see [45]).

Despite the link of t-PA with neurotoxicity, stroke physicians did not alter clinical practice with t-PA as there was no evidence to suggest that t-PA administration actually promoted neurotoxicity when administered to patients with acute ischaemic stroke or for any other thromboembolic condition. Furthermore, the use of t-PA was already restricted to the first 3 h post-stroke onset because of another problem: Indeed, the greatest fear at the time, and still today, was not neurotoxicity, but rather the ability of t-PA to promote symptomatic intracranial haemorrhage (sICH) (see below).

It is not the purpose of this review to provide a detailed account of the extensive efforts made to unravel the means by which t-PA modulates neuronal function. Nonetheless, it was already well-appreciated that t-PA could directly influence neuronal function, at least in part by its capacity to modulate NMDA receptor activity. There was little argument that t-PA was able to modulate the NMDA receptor [46]. What led to much controversy however, was the mechanism underlying this ability of t-PA to modulate NMDA receptor function. The first direct association of t-PA with NMDA function was provided by Denis Vivien’s group in 2001 where it was reported that t-PA caused an increase in NMDA-mediated calcium influx via proteolytic cleavage and activation of the NR1 subunit of the NMDA receptor [47]. This publication also caused controversy when it was subsequently revealed that the antibodies used to detect the NR1 fragment also cross-reacted with plasminogen [48] and therefore challenged the initial suggestion in this paper that NR1 cleavage actually occurred. Later studies also cast doubt as to whether t-PA could directly (i.e. independently of plasmin) cleave the NR1 subunit given that plasmin was itself shown to cleave NR1 [48]. This was also reported in a later study [49] where plasmin itself, but not t-PA, could cleave NR1 but also required an LDL receptor in this process. While other studies failed to find evidence for NR1 cleavage [48,50], subsequent evidence was provided by Vivien’s group that NR1 cleavage occurred in vivo [51] and was further increased by t-PA administration [52]. Moreover, antibodies were developed that were reported to block t-PA-mediated NR1 cleavage and were furthermore shown to be of therapeutic benefit in models of ischaemic [53] and haemorrhagic stroke [54]. Whether the therapeutic benefit reported using these anti-NR1 antibodies was actually via the blockage of NR1 cleavage is still not clear. While there is little doubt that t-PA engages the NR1 subunit, it is the view of this author that the role or otherwise of NR1 cleavage by t-PA needs to be independently validated.

These studies nonetheless helped set the stage for other research investigating the relationship between t-PA and NMDA receptors in various CNS paradigms. In addition to NR1, t-PA was also shown to interact with other NMDA receptor subunits including NR2-D [55], and NR2B [56]. Adding even further complexity to this topic, a recent study reported that t-PA could actually suppress, rather than increase, NMDA-mediated increase in calcium flux, but only when NMDA was used at relatively low concentrations [57]. Hence, it seems likely that t-PA may modulate the NMDA receptor in different ways.
depending on the circumstances, via either plasmin-dependent [49] or -independent [58] mechanisms.

t-PA, plasmin and the Blood-Brain Barrier: relevance to ischaemic stroke

Long before any relationship was found between t-PA and the CNS, early therapeutic use for t-PA for non-CNS related thromboembolic conditions was known to carry a risk for the development of symptomatic intracerebral haemorrhage (sICH). Patients administered t-PA for myocardial infarction [59] or for pulmonary embolism [60] have been reported to display a frequency of t-PA-induced sICH between 1 and 1.5%. Hence, even in clinical conditions where there is, apparently, no evidence for brain ischaemia, t-PA was still shown to induce alterations in the brain microvasculature that in turn promoted cerebral bleeding. This damaging effect of t-PA was presumed to reflect proteolytic damage to the extracellular matrix (ECM) comprising the brain microvasculature. Indeed, it has been known for decades that proteases, including the plasminogen activators and the matrix metalloproteinases (MMPs) were critical mediators of ECM turnover in a general sense, and this was likely to be reflected at the level of the blood-brain barrier (BBB). Initial reports on the influence of proteases on the BBB focused mostly on the role of the MMPs; indeed MMP-9 was shown early on to be upregulated in models of focal cerebral ischaemia and linked with BBB breakdown [61]. Evidence is still accumulating to further suggest a causal role of the MMPs (including MMP-3) at promoting BBB breakdown in cerebral ischaemia [62] as recently reviewed [63].

One of the first studies directly implicating t-PA with BBB permeability was reported in 2002 in a rat model of ischaemic stroke where intracisternal delivery of the t-PA specific inhibitor, neuroserpin, reduced the deleterious (neurotoxic) effects of intravenously (i.v.) delivered human t-PA. This protective action of neuroserpin also reduced the degree of t-PA-mediated increase in BBB permeability [64]. Further evidence supporting the notion that the plasminogen activating system was critical in promoting BBB permeability was published by Dan Lawrence’s group in 2003 where t-PA was shown to increase BBB permeability independently of plasmin generation, and, interestingly, independently of MMP-9 activation. Moreover, this group revealed a critical role for the LDL receptor, LRP-1 in this process [65]. In a subsequent study by the same group, t-PA was shown to promote BBB permeability via direct (i.e. plasmin-independent) cleavage and activation of PDGF-CC [66]. It was further demonstrated that the protein tyrosine kinase inhibitor, imatinib (‘Gleevec’), that works downstream of the PDGF-CC activated PDGFRα1 receptor in perivascular astrocytes, inhibited the ability of t-PA to open the BBB [66]. This striking finding has recently led to clinical trials testing the effectiveness of imatinib in patients with ischaemic stroke, with positive preliminary findings [67].

Notwithstanding the elegant results linking t-PA-mediated, yet plasmin-independent BBB opening via the PDGF-CC pathway, a number of plasmin-dependent pathways have also been reported. Exogenous t-PA was shown to increase BBB permeability via plasmin-mediated cleavage of monocyte chemoattractant protein-1 (MCP-1) in vitro and in wild-type mice [68], and in models of ischaemic stroke via activation of Factor XII (and subsequent activation of plasma kallikrein [69]) and more recently via activation of complement C3 [70]. Other groups also reported a dependence of plasminogen for exogenous t-PA to increase BBB permeability in mouse models of ischaemic stroke [71]. It was also shown in a mouse model of hyperfibrinolysis, that transgenic overexpression of t-PA in liver (that resulted in an increase in plasma levels of t-PA) increased BBB permeability in a plasmin- and bradykinin-dependent mechanism [72]. Taken together, t-PA can increase permeability of the BBB under non-injurious conditions, and in pathological circumstances via different pathways that may or may not require plasmin formation. What remains to be determined is the temporal relationship between t-PA, plasmin and their various substrates and downstream signalling pathways that act upon the BBB.

Regardless of the sequence of events involved in the process of BBB opening by t-PA, a more fundamental question is the physiological purpose for this effect of t-PA on the BBB in the first place. The above discussion has been grouped under a general topic of BBB permeability. Perhaps this needs to be better defined and in doing so enable a more targeted discussion of the role of t-PA at this prime location. As discussed in a recent extensive review of this topic [73], the neurovascular unit that incorporates the BBB, responds to changes in cerebral blood flow (‘neurovascular coupling’), movement of molecules across the BBB (‘neurobarrier coupling’) and changes in local metabolic factors (‘neurometabolic coupling’). t-PA has been linked to all of these processes [73] but not necessarily in a detrimental sense. t-PA has been reported to promote glucose uptake in the damaged brain following ischaemia [74] and more studies have accumulated reporting neuroprotective effects for t-PA [75–77]. After all, it stands to reason that the capacity of t-PA to modulate these important roles of the BBB was not designed to cause harm. Nonetheless, it cannot be denied that under the experimental conditions reported in the literature, that the presence of t-PA can indeed promote harm particularly at high concentrations, and importantly that the absence of t-PA (i.e. as in t-PA−/− mice) is neuroprotective in models of brain injury.

Although the overall weight of opinion today is that the action of t-PA at the BBB fulfils an important physiological requirement, as with all biological processes, tight regulation is essential. It could easily be argued that
during the therapeutic administration of t-PA in conditions of acute ischaemic stroke, and also for myocardial infarction and pulmonary embolism, when plasma t-PA levels increase transiently many hundred-fold, that these normal regulatory processes are overwhelmed allowing unabated access of t-PA to the neurovascular unit, with the end result being over-activation of BBB permeability, causing intracerebral haemorrhage.

t-PA, plasmin and the Blood-Brain Barrier: relevance to Traumatic brain injury

The presence of t-PA and other components of the plasminogen activating system within the CNS led to questions addressing the relationship between tPA in Traumatic Brain Injury (TBI), a condition where both excitotoxicity [78] and BBB disruption [79] are also prominent. BBB disruption in TBI can be a critical event leading to cerebral oedema and increased intracranial pressure [80]. The first association between t-PA and TBI was provided in 2001 by Mori et al. [81] where it was reported that t-PA deficiency resulted in reduced oedema and promoted a faster recovery in a mouse model of TBI, while intravenous administration of t-PA to pigs subjected to a fluid percussion injury model of TBI resulted in increased brain water content [82]. Endogenous levels of t-PA were also reported to increase ~30% in the ipsilateral brain in mice within 3 h of injury as determined using a controlled cortical impact model of TBI [41]. Later studies evaluating changes in BBB permeability following TBI in t-PA<sup>−/−</sup> mice and in T4 transgenic mice neuronally overexpressing t-PA, also resulted in changes consistent with a potentiating effect of t-PA on BBB permeability; i.e. t-PA<sup>−/−</sup> mice had reduced extravasation of plasma proteins into the damaged brain, whereas the opposite was seen in the transgenic T4 mice [83]. What however, was surprising in this paper was the mechanism underlying the potentiating effect of t-PA. Here it was reported that this ability of t-PA to increase BBB permeability relied upon the formation of complexes formed between t-PA and its primary inhibitor, PAI-1. tPA:PAI-1 complexes once formed promoted BBB opening by binding to LDL receptors. Direct intra-cortical injection of preformed tPA:PAI-1 complexes into t-PA<sup>−/−</sup> mice following TBI caused a significant increase in BBB permeability, while injection of complexes into the CSF of naive (uninjured) wild-type mice also increased BBB permeability [83]. Whether complex formation between t-PA and other serine protease inhibitors (‘Serpins’) i.e. tPA: neuroserpin, tPA:protease nexin-1 or tPA:PAI-2 complexes, also produce similar effects in TBI or if this is a unique property of the tPA:PAI-1 complex still remains to be determined. It is well known that tPA:Serpin complexes are cleared via LDL receptors with different affinities and internalisation rates suggesting that differences could indeed exist. Furthermore, since intracisternal delivery of neuroserpin was shown to reduce the extent of t-PA-mediated neurotoxicity in models of ischaemic stroke [64], it may well be that tPA:PAI-1 complexes have a unique capacity to increase BBB permeability. The observation of tPA:PAI-1-driven BBB opening also had clinical relevance as elevated levels of tPA:PAI-1 complex were found in the CSF of patients with severe TBI and that levels of these complexes in the CSF of trauma patients positively correlated with injury severity [83].

While tPA:PAI-1 complex formation can certainly drive BBB opening in TBI, the PDGF-CC mediated pathway is also pertinent in this condition. As previously reported in models of ischaemic stroke [66], administration of imatinib also effectively reduced extravasation of plasma proteins into the parenchyma of mice subjected to TBI. Moreover PDGF-CC levels were also detected in the CSF, and similarly correlated with injury severity [84].

Another prominent signalling pathway activated by t-PA in vitro and in vivo and involved in the promotion of BBB permeability is the Rho-kinase (ROCK) signalling pathway [85–87], notably ROCK-2 which may be downstream of LDL-receptor engagement [88]. It seems likely therefore, that common pathways are initiated by t-PA to modulate the BBB in both TBI and ischaemic stroke: one that is reliant upon protease activity (either via plasmin generation or by direct action of t-PA on other substrates, i.e. PDGF-CC, MCP-1, Factor XII), and one not related to proteolytic activity at all, but rather by a receptor-mediated process.

What surprises this author, however is that inhibition of one particular pathway can have such a profound role at blocking t-PA-mediated increase in BBB permeability, when all of the other pathways remain seemingly intact. If all pathways are activated concurrently, how can the disruption of one pathway be so revealing? Perhaps a more realistic explanation is that the various pathways identified may have distinct temporal roles, i.e. some pathways may be more critical at regulating the BBB during initial events post-brain injury, while other pathways become more pertinent at later stages, but this still remains an open question.

Figure 1 provides a schematic overview of the plasmin-independent and plasmin-dependent pathways utilised by t-PA in the modulation of BBB permeability.

The influence of the plasminogen activating system on the immune response: relevance to stroke and TBI

While the main focus of this review is the relationship between the plasminogen activating system and the CNS, it is important to highlight the marked effect of this system on the modulation of the immune and inflammatory responses, since these influences also have a bearing in the CNS and in the host response to brain injury. The roles of t-PA/plasmin in inflammation have been

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Fig. 1. t-PA promotes BBB permeability via plasmin-independent and plasmin-dependent pathways. t-PA can modulated blood-brain barrier permeability independently of plasmin via PDGF-CC activation [66,84] or via complex formation with PAI-1 [83]. PDGF-CC can also be activated by plasmin [120] (dotted line) but the contribution of plasmin-mediated activation of PDGF-CC during BBB opening remains unknown. t-PA-mediated plasmin generation can also increase BBB permeability via activation of matrix metalloproteinases (i.e. MMP-9 and MMP-3) [62,71], MCP-1 [68], Factor XII (that in turn activates kallikrein [69]), or by activating bradykinin B2 receptor antagonists or by C3a receptor antagonists. t-PA-mediated increase in BBB permeability also requires activation of the Rho Kinase (ROCK) pathway and can be inhibited by ROCK antagonists including fasudil [87] or the ROCK 2 inhibitor, KD025 [88]. Imatinib effectively blocks t-PA initiated PDGF-CC dependent signalling. The LDL receptor (LDLR) antagonist, RAP, can block both plasmin-dependent and independent pathways.

recognised for many years (recently reviewed in [89]), as has the role of this system on the innate immune response [90,91] and the complement pathway [92] (reviewed in [93]). Plasmin has generally been described as being pro-inflammatory, and to promote phagocytosis in macrophages [90]. Furthermore, plasmin generated on the surface of necrotic cells, due to the lysine-dependent recognition of misfolded proteins by plasminogen and t-PA [94], also enhanced their phagocytic uptake by human dendritic cells (DCs) and by diverse mouse DC sub-types in vitro and in vivo [95,96]. However, plasmintreated DCs maintained an immature phenotype displayed reduced migration to lymph nodes, increased the release of the immunosuppressive cytokine TGF-β, and lost their capacity to mount an allogeneic response [91] strengthening the notion that plasmin itself promotes an immunosuppressive state. An immunosuppressive phenotype is also well known to accompany CNS injury, including ischaemic stroke, TBI and spinal cord injury [97]. A 2016 publication in Nat Med has provided evidence for post-stroke immunosuppression in humans and also in mouse models of ischaemic stroke. What was notable in this study however, was that the increase in infection rates post-stroke were due to translocation of commensal gut bacteria to the lung and, based on mouse studies in this paper, was a consequence of gut epithelial barrier disruption [98]. A similar dysfunction in gut permeability has also been described in TBI [99]. Since t-PA, via plasmin generation, has also been reported to increase gut-epithelial barrier permeability [100], is it possible that the administration of t-PA for patients with ischaemic stroke, might intensify post-stroke immunosuppression, promoting a greater risk of infection? Could endogenous t-PA function in a similar capacity in TBI? Interestingly, a recent clinical study found no evidence of improved outcome in patients with acute ischaemic stroke treated with antibiotics: ‘the Preventive Antibiotics in Stroke Study, PASS’ [101]. However, post hoc analyses revealed that patients who received intravenous thrombolysis with t-PA, did indeed have improved outcome if also administered the antibiotic (ceftriaxone) as compared with the control group not administered t-PA [102]. While this latter study requires confirmation in a larger cohort, these findings are consistent with the notion that t-PA administration can promote an immunosuppressive environment that may exacerbate infection risk in patients with ischaemic stroke.

While the discussion above points to an immunosuppressive role for plasmin, other studies have reported that plasminogen is actually required to implement a full neutroinflammatory response [103,104]. This clearly deviates from the current discussion and indicates that plasmin (ogen) may therefore function in different capacities with regards to inflammation and the immune response that may depend on the type of insult, magnitude and location. Clearly more studies are needed in this growing and important area.

**Severe trauma and TBI: the paradoxical effect of anti-fibrinolytic agents**

While administration of t-PA may impact on the immune response, one can consider an opposite scenario in severe trauma, including TBI. In such clinical conditions, anti-fibrinolytic agents are used for the sole purpose of reducing bleeding. The clinical benefit of the use of anti-fibrinolytic drugs in trauma was highlighted in the 2010 CRASH-2 trial [105], although this study has been the subject of much scrutiny i.e. [106] among others. The anti-fibrinolytic agent of choice is the lysine analogue, tranexamic acid (TXA) that works by binding to lysine binding sites in plasminogen, thereby blocking the capacity of plasminogen to interact with exposed lysine residues on the fibrin surface. Many non-fibrinolytic effects of plasmin are known some of which have been highlighted in this review and elsewhere (see [107] for further detail). Hence it is likely that plasmin inhibition may have consequences unrelated to haemostasis. For example, TXA has been shown to block the ability of t-PA to increase BBB permeability in vitro [87,108], while studies in patients...
undergoing cardiac surgery have reported marked effects of TXA or other antifibrinolytic agents (i.e. aprotinin) on the inflammatory response [109–111]. A recent publication also reported reduced lung barrier permeability by TXA in a rat model of polytrauma [112], while TXA was also recently reported to reduce ‘endothelialopathy’ of trauma and shock in an in vitro model [113]. Hence it is reasonable to suggest that the blockade of plasmin generation by TXA in coagulopathic trauma patients, or indeed in other conditions associated with severe bleeding, may also have unintended consequences on outcome [107]; such effects could even be beneficial and may have contributed to the beneficial effects reported with TXA administration in the CRASH-2 trial. However, there is also a potential for TXA to surprisingly promote bleeding in situations associated with high levels of urokinase. This so-called ‘TXA paradox’ [114] occurs because of a conformational change in plasminogen following the binding by TXA that in turn increases its ability to be selectively activated by urokinase [115,116]. Hence, the unanticipated increase in bleeding seen in severe trauma patients administered TXA at later time points (>3 h) in the CRASH-2 trial, may be due to increases in u-PA levels (inferred from TBI models in mice [117]). Moreover, marked regional differences in plasmin activity in pigs administered TXA have been reported. TXA treatment resulted in a dramatic increase baseline plasmin activity in the kidney [118], an organ that expresses high levels of urokinase. Whether this has any clinical consequences in trauma affecting the kidney remains to be determined.

Conclusion

It is now patently clear that the plasminogen activating system is not solely directed at removing fibrin deposits and blood clots. While it has long been associated with the modulation of the inflammatory and immune responses, this system is now well cemented into many important areas related to brain function and dysfunction. Although this initially took the field by surprise, it has also raised prospects for new opportunities and insights for the treatment of ischaemic stroke, brain injury and more recently for neurodegenerative conditions [119].

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The author states that he has no conflict of interest.

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