Review Article

Tissue plasminogen activator and inflammation: from phenotype to signaling mechanisms

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Abstract: In disease conditions, inflammatory cells, such as neutrophils, T cells, and monocytes/macrophages, are recruited in response to injury cues and express panoply of proinflammatory genes through a combination of transcription factors. Tissue plasminogen activator (tPA), a member of the serine protease family, has been shown to act as cytokine to activate profound receptor-mediated signaling events. In this review, we will discuss the role of tPA in inflammation in various models, and illuminate its signaling mechanisms underlying its modulation of inflammation.

Keywords: Tissue plasminogen activator, tPA, inflammation, phenotype, signaling mechanisms

Introduction

One of the notable histological hallmarks of inflammation is the accumulation of inflammatory cells at the site of injury. In response to injury, such as tissue damage or infection, inflammatory cells including neutrophils, monocytes/macrophages, and T cells are recruited to the injury sites. These cells not only play an important role in the clearance of pathogens, but also express a panoply of pro- and anti-inflammatory genes through a combination of transcription factors such as NF-κB, which regulates the induction, progression, and resolution of inflammation [1]. Excessive response will cause inflammatory disease and result in tissue destruction and fibrosis. tPA is a member of the serine protease family that plays a pivotal role in the homeostasis of blood coagulation/fibrinolysis and matrix regulation [2-5]. tPA has been shown to modulate the inflammatory response to tissue injury in various models [6, 7]. Recent studies demonstrate that tPA also acts as a cytokine to trigger profound receptor-mediated intracellular signaling events [2, 8-11]. In this review, we will discuss the role of tPA in inflammation, as well as its underlying signaling mechanisms.

The structure of tPA

tPA is a 69-kDa glycoprotein consisting of 527 or 530 amino acids. It is synthesized within cells and released as a single chain enzyme, which is subsequently cleaved by plasmin into a two-chain form (heavy chain and light chain). The single-chain tPA contains 4 domains: 1) a finger (F) domain, which is homologous to the first domain of the fibronectin; 2) an EGF domain, which is homologous to EGF; 3) two kringle (K) domain; and 4) the catalytic protease (P) domain. The heavy chain of two-chain tPA contains F, EGF, and K domains, whereas, the light chain contains the P domain. The active site of tPA consists of Histidine 322, Asparagine 371, and Serine 478 [12]. The single mutation of Serine 478 to Alanine renders tPA catalytically inactive, while its other function remains intact [13]. This mutant non-enzymatic tPA plays an instrumental role in identifying the protease-independent effects of tPA.

tPA is a hybrid molecule with dual functions: protease and cytokine

Although tPA is generally considered as a potent protease belonging to the serine protease family, mounting evidence demonstrates that tPA
also possesses various functions that are discrete from its proteolytic activity in a variety of physiological and pathological settings [14, 15]. Our recent studies demonstrate that tPA is actually a molecule with dual functions [2, 8-10, 16]. As a serine protease, tPA plays a pivotal role in the homeostasis of blood coagulation/fibrinolysis and extracellular matrix regulation [2]. As a cytokine, tPA executes multiple actions by binding to its membrane receptors and triggering profound intracellular signaling events [2, 8-11, 16]. This conceptual advance provides the ground for the explanation of many once bewildering functions of tPA [2, 17-22].

**tPA receptors: LDL receptor-related protein-1 (LRP-1) and annexin A2**

tPA does not have a dedicated and specific receptor. However, extensive studies have identified candidates that act functionally and biologically as tPA receptor by initiating intracellular signaling and mediating downstream cellular responses. There are at least two known receptors for tPA. One, the most known receptor, is LRP-1, which was originally identified as a tPA receptor on hepatocytes [23]; the other is annexin A2, which was initially found on microglia [24].

LRP-1, also known as α2-macroglobulin receptor (α2MR) [23] or type V TGF-β receptor (TβR-V) [25], is a member of the LDL receptor family that is implicated in lipoprotein metabolism and in the homeostasis of proteases and protease inhibitors [26, 27]. Expression of LRP-1 is ubiquitous. Mature LRP-1 consists of an extracellular 515-kDa α subunit and an 85-kDa β subunit with a transmembrane segment and cytoplasmic tail containing two NPxY motifs and numerous tyrosine residues [26, 28, 29]. Phosphorylation of the tyrosine residue(s) is essential for LRP-1 to relay its signal, though the exact mechanisms of the phosphorylation remain not complete understood. We have shown that tPA induces the phosphorylation of LRP-1 Tyr 4507, which is indispensable to tPA-mediated fibroblast proliferation [10]. There are four putative ligand-binding domains in the extracellular region of LRP-1; each of them contains two to eleven individual cysteine-rich repeats [26]. The domains II and IV in the LRP-1 have been identified as the binding sites for tPA [26, 30].

Annexin A2 is a member of the Ca$^{2+}$- and phospholipid-binding protein family. It has a unique structure that allows it to dock onto membrane in a peripheral and reversible manner [31]. Annexin A2 has been identified as a major membrane receptor of tPA on endothelial [32], microglia cells [33], and other cancer cells [34]; and is implicated in mediating certain signal transductions [20, 33, 35]. The core domain in the C terminus of annexin A2 consists of four highly α-helical annexin repeats which mediates its membrane binding [31]. tPA has been shown to bind to the hexapeptide LCKLSL (residues 7-12) in the N terminus of annexin A2 [36]. However, unlike the other well-known tPA receptor, LRP-1, annexin A2, as a membrane-associated protein, lacks the transmembrane domain, to which tPA can only dock [31, 34]. Recently, we demonstrated that under the stimulation of tPA, annexin A2 aggregates with integrin CD11b, leading to the activation of integrin-linked kinase (ILK) pathway [16]. Thus annexin A2 can transduce tPA signaling through its interaction with integrins.

**Role of tPA in inflammation: studies from animal models**

tPA has been shown to modulate inflammatory infiltration in numerous disease models [6, 7, 16, 37]. In an acute brain injury model, tPA was shown to mediate F4/80 macrophage accumulation and activation in the ischemic brain [37]. In a carbon-tetrachloride-induced liver fibrosis model, tPA-deficient mice demonstrated decreased infiltration of T cells in comparison with their wild-type counterparts [6]. Moreover, it was found that tPA promoted infiltration of macrophages or other leukocytes in both models of acute [7] and chronic kidney injury [16]. Thus, tPA appears to have broad implication in the modulation of infiltration and inflammation in diverse organs. Intriguingly, the increased inflammatory infiltration in most of those models is accompanied by the concomitant induction of tPA [8, 9, 17, 38], suggesting that tPA may be a common endogenous factor that modulates inflammatory infiltration and response in multiple organ systems.

**tPA and cerebral inflammation**

Zhang and Yepes group studied the role of tPA in microglial activation in an ischemic brain injury model [37]. Middle cerebral artery occlusion...
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MCAO was induced in the wild-type, tPA knockout (tPA-/-), and microglia-specific LRP-1 knockout (macLRP-/-) mice. They found that MCAO induces microglial activation, as demonstrated by ameboid morphology and double immune staining of β-isolectin and F4/80, as well as inflammatory markers such as iNOS, in the wild-type mice. However, MCAO-induced microglial activation is significantly decreased in tPA-/- and macLRP-/- mice. Intriguingly, administration of exogenous tPA increases microglial activation in tPA-/- mice but not in the macLRP-/- mice, suggesting that LRP-1-mediated tPA cytokine function induces microglial activation and inflammatory response in the ischemic brain. The exact signaling mechanism of tPA effect remains unknown. But tPA has been shown to promote LRP-1-mediated NF-κB signaling in the same ischemic brain injury model [39].

**tPA and liver inflammation**

In a carbon-tetrachloride-induced liver fibrosis model, Higazi A, et al demonstrated that carbon-tetrachloride induces liver fibrosis, inflammatory infiltration, and increased serum level of IFN-γ, IL-4 and IL-10 in wild-type mice [6]. Flow cytometry analysis indicated that the intrahepatic infiltration mainly consists of CD4 and CD8-positive T cells. However, tPA-/- mice displayed significantly decreased fibrosis, intrahepatic infiltration of T cells, and lower level of cytokines.

**tPA and renal inflammation**

tPA has been shown to promote renal inflammation in both models of acute and chronic kidney injury. In a renal ischemia-reperfusion injury model, Roelofs, et al found that tPA-deficient mice display significantly less neutrophil influx into the interstitial region and quicker recovery of renal function than wild-type mice [7]. In addition, wild-type mice treated with tPA-antisense oligonucleotides show less histological damage, better renal function, and less neutrophil influx than the control mice. Of note, these tPA effects are independent of complement C3 and plasmin activity. In the obstruction-induced chronic kidney injury model [16], we demonstrated that obstruction-induced CD11b-positive macrophage infiltration, NF-κB activation, and proinflammatory chemokine expression are attenuated in tPA-deficient mice. Moreover, the interstitial cells with activated NF-κB are largely CD11b-positive macrophages, indicating a possible important role of macrophages in tPA-mediated NF-κB activation. The different types of infiltrating cells in the acute and chronic kidney injury models may reflect the temporal order of inflammatory recruitment in response to injury.

**tPA and bacterial infection**

The role of tPA in bacterial infection has been investigated in both kidney and lung infection models. In an E. coli-induced acute pyelonephritis model [40], it was found that tPA-/- kidneys contain significantly higher number of bacteria, as well as higher levels of IL-1β and TNF-α. The ability of tPA-deficient neutrophils to generate oxidative burst reaction and eliminate E. coli is dramatically impaired. In line with this observation, Renckens R, et al demonstrated that over-expression of tPA markedly improves host defense against K. pneumonia-induced pneumonia as indicated by less bacterial growth and dissemination, reduced distant organ injury, and decreased mortality [41]. Since innate immunity plays a fundamental role in the host defense against pathogens, these studies indicate that tPA may also play an important role in the modulation of innate immunity.

**tPA and inflammation: mechanistic studies**

**tPA and NF-κB activation**

NF-κB activation is a central event in the initiation and progression of inflammation. The members of the NF-κB family of transcription factors exist in form of homo- and heterodimers that formed among the five members of NF-κB family: p50, p52, p65 (RelA), RelB, and c-Rel. In physiological status, NF-κBs are retained in the cytoplasm by its specific inhibitor, IκB [42, 43]. Upon activation, IκB will be phosphorylated, which leads to its degradation, resulting in the release and nuclear translocation of p65/p50 (canonical activation) or RelB/p52 (non-canonical activation), and subsequently DNA binding and gene transcription [42, 43]. Role of tPA in NF-κB activation is context dependent [16, 39]. tPA may execute cell type-specific biological functions by binding to different membrane receptors (LRP-1 or annexin A2) and initiating different intracellular signaling events.
We investigated the role of tPA in NF-κB activation, and found that tPA activates NF-κB pathway and induces the expression of the associated proinflammatory chemokines in macrophages through a novel signaling cascade involving annexin A2-mediated aggregation of integrin CD11b and the subsequent activation of ILK pathway [16]. We further demonstrated that annexin A2 mediates tPA-induced NF-κB activation in macrophages; and it induces the interaction and aggregation of annexin A2 with CD11b, leading to the clustering and activation of integrin CD11b signaling, which in turn activates its downstream ILK and phosphorylates IκB. Phosphorylation of IκB leads to its degradation and the release of NF-κB dimers into nuclei, resulting in the subsequent DNA binding and transcription of target proinflammatory genes (Figure 1B). Blockade of any step within this signaling cascade by various strategies, such as annexin A2 and ILK siRNAs, CD11b knockout or neutralizing antibody, or infection with dominant-negative ILK-kd or C-terminal ILK adenoviral vectors, eliminates tPA-induced NF-κB activation. We also found that tPA modulates NF-κB signaling in vivo, because obstruction-induced phosphorylation of p65 and chemokine expression are dramatically reduced in the kidneys from tPA-deficient mice, accompanying with decreased collagen deposition and macrophage infiltration.

In contrast, Zhang X, et al demonstrated that tPA and LRP-1 mediates cerebral ischemia-induced NF-κB activation (Figure 1A) [39]. They found that ischemic insult induces NF-κB acti-
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vation, which is attenuated after LRP inhibition or tPA knockout. Exogenous administration of tPA into the tPA/- mice restores the level of NF-κB activation comparable with that in wild-type mice. However, tPA/- mice received intracerebral injection of tPA in combination with RAP, a specific LRP-1 antagonist, display similar level of NF-κB activation in comparison with tPA-deficient mice. These finding indicate that LRP-1 mediates tPA-induced NF-κB activation in brain.

tPA and macrophage motility

Macrophages play an important role in the initiation and modulation of inflammation. Enhanced motility is one of the main contributing factors that lead to the recruitment and accumulation of macrophages around the site of injury. Cao, et al investigated the role and the underlying mechanism of tPA in the regulation of macrophage motility [44]. They demonstrated that LPS-induced peritoneal macrophase efflux is attenuated in the tPA-deficient mice, and genetic inactivation of integrin Mac-1, tPA, PAI-1 or LRP-1 abrogates macrophase migration. Further investigations showed that tPA forms complex with LRP-1, Mac-1, and PAI-1, which is essential to macrophase migration (Figure 1C). We recently found that tPA activates FAK and Rac-1 signaling, which is known to play an important role in cell motility, in macrophages (unpublished data), suggesting that the integrin downstream signaling through FAK/Rac-1 may mediate tPA-induced macrophage migration.

tPA and inflammation: future perspective

Although numerous studies clearly indicate that tPA play an important role in the modulation of inflammation. There are still many gaps in our understanding of the molecular mechanisms underlying the actions of tPA need to be filled, such as the subsets of tPA-mediated infiltrating macrophages and T cells, the downstream signaling mechanisms of tPA-promoted macrophage motility, and the role and mechanism of tPA in the interaction with the signaling mediators within innate immunity. Future investigations towards the above aspects will eventually lead to the development of specific treatments targeting the autoimmune and inflammatory diseases.

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Disclosure of conflict of interest

None.

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