


REVIEW

# A critical role for plasminogen in inflammation

Sarah K. Baker  and Sidney Strickland 

**Plasminogen and its active form, plasmin, have diverse functions related to the inflammatory response in mammals. Due to these roles in inflammation, plasminogen has been implicated in the progression of a wide range of diseases with an inflammatory component. In this review, we discuss the functions of plasminogen in inflammatory regulation and how this system plays a role in the pathogenesis of diseases spanning organ systems throughout the body.**

## Introduction

Plasmin, primarily a blood protein, plays a variety of physiological roles in the mammalian body. The plasminogen activator system consists of a proteolytic cascade of serine proteases, binding proteins, and inhibitors that control the temporal and spatial generation of active broad-spectrum serine protease plasmin (Chana-Muñoz et al., 2019). This system has been studied for its physiological roles including fibrinolysis, wound healing, cell signaling, and extracellular matrix (ECM) degradation (Li et al., 2003). There are both direct and indirect ways that plasminogen and related proteins contribute to inflammation, and there has been a recent focus on the role that plasminogen has in the regulation, development, and progression of diseases with an inflammatory component. This review focuses on the important function of plasminogen and plasmin in inflammation and discusses how plasminogen contributes to disease pathogenesis.

## Overview of the plasminogen activator system

Plasminogen is mainly produced in the liver and then released into the circulation (Raum et al., 1980). Activation of plasminogen leads to the generation of plasmin, a broad-spectrum serine protease. There are two major plasminogen activators that are also serine proteases, tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA). The two activators act via different mechanisms in differing compartments to activate plasminogen. tPA has a primary role in fibrinolysis, acting on polymerized fibrin, whereas uPA primarily localizes to the surfaces of cells such as neutrophils and macrophages for functions such as ECM remodeling or monocytic cell migration (Castellino and Ploplis, 2005). uPA usually binds to cell surfaces using the high affinity uPA receptor (uPAR). Both tPA and uPA are inactivated by the serine protease inhibitor (serpin) PAI-1. PAI-2 and nexin are also regulators of tPA and uPA activity (Castellino and Ploplis, 2005). Plasmin is inhibited by  $\alpha_2$ -antiplasmin (Hudson, 2017). This network is summarized in Fig. 1.

A recent article reviewed the evolutionary origins of the plasminogen activator system (Chana-Muñoz et al., 2019), suggesting that plasminogen, tPA, uPA, and PAI-1 emerged from a single common gene, which then duplicated and further diverged into the plasminogen activator system proteins. The authors show that hepatocyte growth factor, lipoprotein(a) (Lp(a)), and macrophage stimulating 1 are evolutionarily similar to plasminogen, whereas hyaluronan-binding protein and hepatocyte growth factor activator are evolutionarily similar to tPA and uPA. While these related proteins have diverse roles, they are generally involved with vascular functions and inflammation, similar to plasminogen (Chana-Muñoz et al., 2019). Inflammatory proteins are mediators of coagulation processes, and these data suggest that coagulation and innate immune proteins evolved together to modulate these physiological processes.

## Human plasminogen deficiency

Plasminogen deficiency in humans is rare and leads to several well-characterized pathologies. Low levels of plasminogen commonly lead to ligneous conjunctivitis characterized by thick, woody, fibrinous deposits on the body's mucosal membranes (Mingers et al., 1997). These deposits are commonly found on the inside of the eyelids but can also affect other mucosal membranes such as the larynx, vocal cords, nose, genitourinary tract, peritoneum, and gingiva (Mehta and Shapiro, 2008).

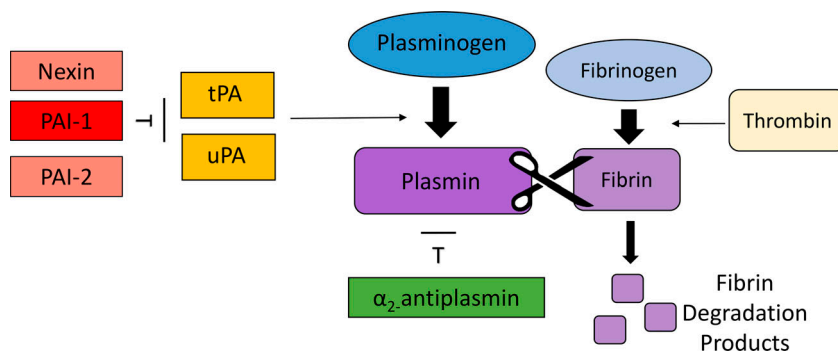
Plasminogen deficiency can be quantitative (type I, decreased plasminogen production) or qualitative (type II, normal protein levels with decreased activity; Mehta and Shapiro, 2008). The first known patient with plasminogen deficiency was reported in 1978. This patient suffered from recurring thrombosis and was found to have normal levels of plasminogen protein with reduced activity of about half the values of normal subjects. Further investigation of the family indicated that other relatives had reduced plasminogen activity, passed on as an autosomal

Patricia and John Rosenwald Laboratory of Neurobiology and Genetics, The Rockefeller University, New York, NY.

Correspondence to Sidney Strickland: [strickland@rockefeller.edu](mailto:strickland@rockefeller.edu).

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**Figure 1. Overview of the plasminogen activator system.** PAI-1, PAI-2, and nexin serve as inhibitors for the plasminogen activators tPA and uPA. Plasminogen is activated by tPA or uPA to form plasmin, which can then be inactivated by  $\alpha_2$ -antiplasmin. The major substrate for plasmin is fibrin, which is formed via the coagulation cascade following cleavage of fibrinogen by thrombin. Plasmin degrades fibrin into fibrin degradation products.

characteristic, with heterozygotes for the abnormal plasminogen having half the normal plasmin activity and one homozygote having basically no plasmin activity (Aoki et al., 1978). This deficiency, along with other deficiencies in plasmin activity, was later characterized as being due to a mutation in the active site of plasminogen, leading to type II plasminogen deficiency (Robbins, 1992). While most plasminogen deficiencies come from genetic alterations, ligneous conjunctivitis was reported following treatment with the plasmin inhibitor tranexamic acid and resolved once treatment was discontinued (Diamond et al., 1991).

### Plasminogen has a variety of functions that lead to inflammatory regulation

As a broad-spectrum protease, plasminogen has many functions that contribute to the regulation of an inflammatory response (Fig. 2). These are each reviewed separately below and include fibrinolysis, complement interaction, ECM degradation, cell migration, and resolution of inflammation.

#### Fibrinolysis

Fibrin is a strong promoter of plasminogen activation, serving as a surface for both plasminogen and tPA binding, which allows for accelerated tPA-mediated cleavage of plasminogen into plasmin. In addition, the fibrin scaffold protects plasmin from being inactivated by  $\alpha_2$ -antiplasmin (Hudson, 2017).

Fibrin acts as a framework for platelets, leukocytes, and fibroblasts to bind, release inflammatory signals, and participate in wound healing (Weisel, 2005). Fibrin can induce production of proinflammatory signaling molecules such as IL-8, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, chemokines, and reactive oxygen species (Altieri, 1999; Qi et al., 1997; Jennewein et al., 2011; Perez and Roman, 1995; Smiley et al., 2001). The  $\alpha_M\beta_2$ -binding motif of fibrinogen is specific to fibrinogen's inflammatory functions and is responsible for fibrin binding to leukocytes to modulate leukocyte adhesion to the endothelium (Flick et al., 2004; Languino et al., 1993). Removal of the  $\alpha_M\beta_2$ -binding motif of fibrinogen protects from inflammatory diseases including arthritis and neuroinflammatory disease (Flick et al., 2007; Adams et al., 2007). Because fibrin is proinflammatory, plasmin-mediated fibrinolysis can lead to the reduction of inflammation by removing fibrin.

However, fibrin degradation products also have inflammatory roles. Fibrin proteolysis by plasmin leads to the formation

of various fibrin degradation products such as fragment E, fragment D, D-dimer, B $\beta$ 15-42, and  $\alpha$  chain fragments (Jennewein et al., 2011). These fragments are known to have proinflammatory and anti-inflammatory effects. For example, fragment E stimulates the production of proinflammatory cytokines IL-6 and IL-1 $\beta$  by peritoneal macrophages (Lee et al., 1999). D-dimer is also proinflammatory and stimulates neutrophil and monocyte activation and IL-6 production (Rao et al., 1994; Robson et al., 1994). In contrast, fibrin fragment B $\beta$ 15-42 has an anti-inflammatory effect, leading to reduced leukocyte accumulation in response to myocardial infarct by binding to vascular endothelial cadherin to prevent leukocyte transmigration across endothelial cell monolayers (Petzelbauer et al., 2005).

In some cases, plasminogen depletion in mouse models of inflammation leads to a reduction in inflammatory signals. However, in many instances, this function of plasminogen is dependent on fibrin and fibrinolysis, with plasminogen regulating inflammatory signals only when fibrin is present (Berri et al., 2013; Silva et al., 2019; Raghu et al., 2014; De Nardo et al., 2010; Jennewein et al., 2011). These instances are discussed in more detail below. It is crucial to note that fibrin is the major substrate for plasminogen, and importantly, loss of fibrinogen rescues the pleiotropic phenotypic effects that genetic plasminogen depletion has on mice. *Plg*<sup>-/-</sup> mice have increased mortality, thrombosis, rectal prolapse, and delayed wound healing, but *Plg*<sup>-/-</sup>/*Fbg*<sup>-/-</sup> rescues these phenotypes and these mice are indistinguishable from *Fbg*<sup>-/-</sup> mice, suggesting that fibrinolysis is the central function of plasminogen (Bugge et al., 1996).

#### Complement interaction

The complement system consists of ~20 proteins that circulate in the blood and tissue fluids that become activated in a cascade to play a critical role in inflammation. This system enhances the ability of antibodies to attack and clear pathogens. There is evidence that plasminogen is a complement inhibitor, and it binds to C5, as well as C3 and its cleavage products, C3b and C3d, via lysine residues (Barthel et al., 2012). Plasmin on the surface of the bacteria *Moraxella catarrhalis* degrades C3b and C5b, which may contribute to this bacteria's resistance to being killed by the host (Singh et al., 2015). In addition, complement inhibitor C4b-binding protein binds plasminogen to create a complex that is present in serum and plasma. Activation of plasminogen

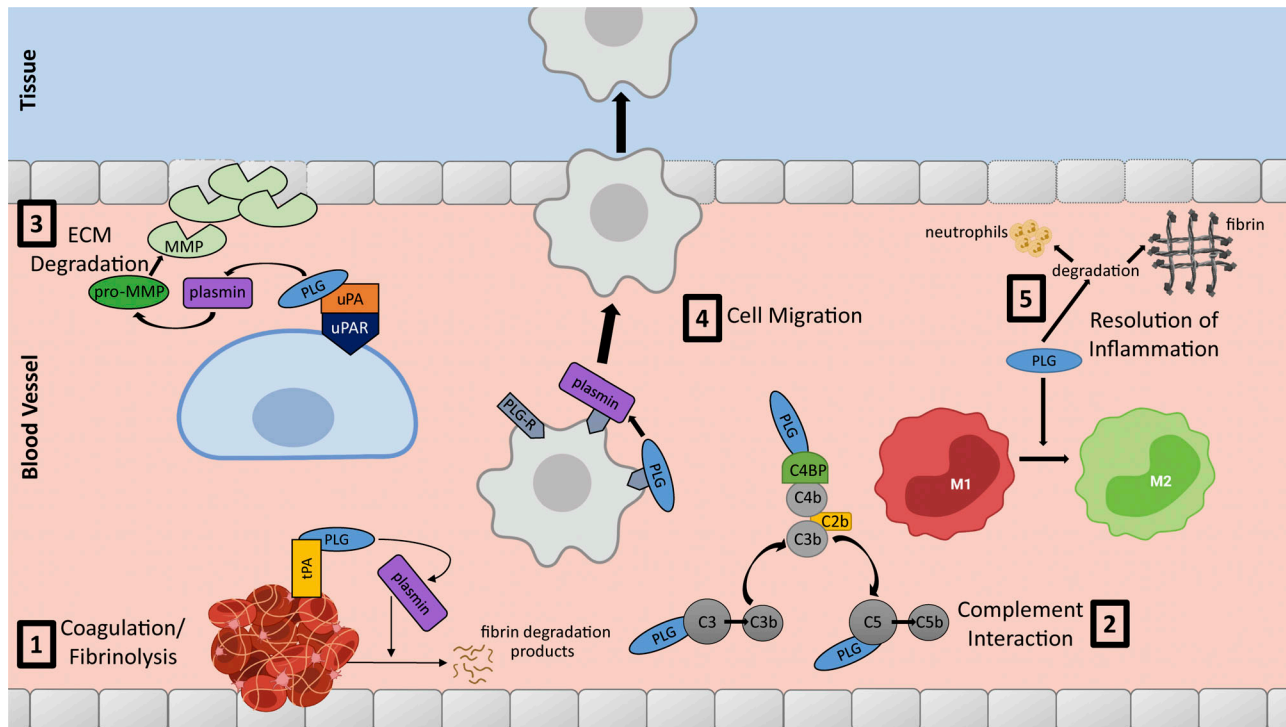


Figure 2. **Overview of the ways plasminogen (PLG) contributes to inflammation.** (1) The major substrate for plasmin is fibrin. Following fibrin clot formation through the coagulation cascade, plasminogen participates in fibrinolysis by binding to the fibrin clot along with tPA. It becomes activated to form plasmin, which will then degrade the clot, resulting in the formation of fibrin degradation products. (2) Plasminogen interacts with several proteins of the complement cascade including C3, C3b, C5, and C4BP, which may aid in the modulation of the complement inflammatory response. (3) Cell surface-bound plasmin aids in ECM degradation by activating MMPs that can degrade collagen and other proteins in the blood vessel wall. (4) Plasmin binds to cells via plasminogen receptors (PLG-Rs), which can lead to migration of leukocytes, neutrophils, monocytes, and macrophages. Note that this function is often fibrin dependent; thus fibrinolysis is an important part of plasmin-mediated cell migration. (5) Plasminogen participates in wound healing by clearing fibrin and neutrophils once they are no longer needed at the injured site. Plasminogen also aids in polarization of M1 (proinflammatory) macrophages into M2 (anti-inflammatory) macrophages during this phase.

by uPA leads to an increase in C4b-binding protein, suggesting that these proteins may interact during acute inflammation (Agarwal et al., 2015).

In about two-thirds of patients with atypical hemolytic uremic syndrome (aHUS), a thrombotic microangiopathy, the complement system gets over-activated and destroys healthy cells. A genetic screen indicated that plasminogen deficiency is common among aHUS patients (Bu et al., 2014). However, a more recent publication argues that plasminogen does not have a direct effect on complement proteins in aHUS and that plasmin only inhibits complement activation at concentrations much higher than normal blood concentrations of plasminogen. Plasminogen did not inhibit complement-mediated lysis of red blood cells or endothelial cells but did prevent platelet aggregation, and this proteolytic activity on thrombi may explain why plasminogen deficiency is seen in aHUS (Hyvärinen and Jokiranta, 2015).

**ECM degradation**

ECM degradation is important to the immune response because the ECM is a barrier that prevents cells and pathogens from migrating out of the blood into other tissues. When proteins in the ECM are broken down, this allows for cell migration and invasion. ECM breakdown is essential to normal physiological

processes such as reproduction, development, and tissue remodeling and is also implicated in a variety of diseases. Plasminogen binds to the ECM and can degrade many different ECM proteins, either directly or indirectly, once activated into plasmin. Through ECM degradation, plasminogen can allow pathogens such as bacteria and parasites or immune cells like macrophages or activated T or B cells to migrate into other tissues of the body (Liu and Shih, 2007).

ECM degradation in cultured human mesangial cells cultured on thin films of Matrigel, imitating the ECM, is concentration dependent on exogenous plasminogen and is blocked by the presence of  $\alpha_2$ -antiplasmin and aprotinin. In addition, a monoclonal antibody against tPA or uPA leads to decreased ECM degradation, whereas a monoclonal antibody against PAI-1 increases ECM degradation up to fourfold (Baricos et al., 1995).

Plasmin can also degrade the ECM indirectly through activation of matrix metalloproteinases (MMPs; Castellino and Ploplis, 2005). MMP-2 and MMP-9 are both important for ECM degradation and are crucial enzymes for collagen degradation (Huang et al., 2014). An inhibitor against MMPs, TIMP-1, partially inhibits ECM degradation in the presence of plasmin (Baricos et al., 1995).

Intraperitoneal thioglycollate injection is an experimental model for peritonitis. Thioglycollate-induced peritonitis elicits

an influx of neutrophils and other immune cells into the peritoneal cavity and is thus a good model to study immune cell migration. *Plg*<sup>-/-</sup> mice show decreased leukocyte migration into the peritoneal cavity due to limited trans-ECM migration and decreased MMP-9 activation, and this deficit can be corrected in *Plg*<sup>-/-</sup> mice with the administration of activated MMP-9 (Gong et al., 2008).

In the brain, plasmin may play a role in neuronal death via ECM interactions. Neurons and the ECM closely interact, and disruption of this interaction by plasminogen-mediated laminin degradation induces hippocampal neuronal death. tPA-KO mice show decreased laminin degradation and a decrease in neuronal death in response to kainate (an excitotoxin) injection into the hippocampus. A similar effect is seen when  $\alpha_2$ -antiplasmin is infused into the brain before kainate injection (Chen and Strickland, 1997). Thus, plasminogen mediates ECM disruption via laminin degradation that may contribute to neuronal death in the presence of excess excitatory amino acids.

### Cell migration

**Leukocyte recruitment.** Plasminogen also has a role in cell migration and recruitment of immune cells to the sites of injury. White blood cells, or leukocytes, are the immune cells of the blood and lymphatic system and are important for an immune response to pathogens. There are several types of leukocytes, including neutrophils, eosinophils, basophils, lymphocytes, and monocytes, all of which have different targets in the blood related to the prevention of bacteria, fungi, parasites, and viruses from harming the body. There is evidence that plasminogen is important for full recruitment of these cells into areas of trauma or disease.

Plasminogen can also aid directly in chemotactic cell migration. Rabbit mononuclear cells and neutrophils isolated from peritoneal exudates respond chemotactically to plasminogen to migrate through pores in a chamber (Ward, 1968). In this study, different types of mononuclear cells were not identified and could have included T cells, B cells, natural killer cells, and monocytes.

Lp(a), a risk factor for cardiovascular disease, is associated with apolipoprotein (apo(a)), a plasminogen-mimic, due to kringle domains homologous to plasminogen. Apo(a) competes with plasminogen and interferes with leukocyte recruitment in a model of inflammation. The Lp(a) and apo(a) pathway impedes plasminogen activation and macrophage recruitment, as well as neutrophil recruitment and neutrophil cytokine release in a model of abdominal aortic aneurism (Huang et al., 1996).

**Monocyte and monocyte-derived cell migration.** Monocytes are leukocytes important to adaptive immunity. These cells patrol the endothelial cell wall, release proinflammatory cytokines in response to pathogens, and differentiate into macrophages to migrate into other tissues (Kratofil et al., 2017). The lungs of WT mice inoculated with two influenza A viruses have a large degree of cellular infiltration and alveolar damage, which is reduced in *Plg*<sup>-/-</sup> mice. This decrease in immune cell infiltration is accompanied by a decrease in proinflammatory cytokine levels in *Plg*<sup>-/-</sup> lungs. This function of plasminogen seems dependent on fibrinolysis, as treatment with anicrod, which

promotes fibrin degradation, leads to increased lung inflammation and elevated cytokine levels. Anicrod treatment in *Plg*<sup>-/-</sup> mice reversed the immune cell recruitment protection and cytokine decrease seen with plasminogen deficiency. Likewise, inhibiting plasminogen binding to fibrinogen using 6-aminohexanoic acid decreased inflammation by blocking plasmin-mediated fibrinolysis. Taken together, these data suggest that plasminogen-mediated fibrinolysis is important for the pathogenesis and inflammation associated with influenza A infections (Berri et al., 2013).

Many of plasminogen's roles in inflammation can also be mechanistically linked to fibrin, which has its own role driving an inflammatory response (Davalos and Akassoglou, 2012; Flick et al., 2004). For example, in *Plg*<sup>-/-</sup> mice there is reduced monocyte, macrophage, and dendritic cell recruitment into the peritoneal cavity in a thioglycollate-induced peritonitis model (Silva et al., 2019). Although a previous study (Gong et al., 2008) suggested that this was due to macrophage retention in the peritoneal wall, this more recent study noticed that fibrin accumulated in the peritoneal wall of *Plg*<sup>-/-</sup> mice and hypothesized that decreased fibrinolysis by plasminogen contributed to the decreased monocytic cell migration. Interestingly, a double knockout of plasminogen and fibrinogen (*Plg*<sup>-/-</sup>/*Fbg*<sup>-/-</sup>) rescued recruitment of macrophages into the peritoneal cavity in response to thioglycollate. These results are bolstered by the fact that experiments were run independently in two laboratories at separate institutions and they had the same experimental findings. In addition, an *in vitro* transwell migration assay that looked at cell migration across a fibrin matrix in the presence or absence of plasminogen further validated the idea that fibrin is necessary for plasminogen-dependent macrophage migration. Cells migrated through the membrane easily in the presence of plasminogen but failed to migrate across a fibrin matrix in the absence of plasminogen. Furthermore, the requirement for plasminogen for migration through fibrin matrices is dependent on the  $\alpha_M\beta_2$ -binding motif of fibrin, which sequesters macrophages in the absence of fibrinolysis (Silva et al., 2019).

**Receptors for cell migration.** As plasminogen has versatile functions, it is no surprise that there are many different plasminogen receptors. Plasminogen can bind to proteins with a C-terminal lysine (Miles and Plow, 1985), which includes membrane-bound proteins, intracellular proteins, nuclear proteins, and integrins (Flick and Bugge, 2017). Cell surface binding is crucial for many of plasminogen's functions except fibrinolysis, when fibrin serves as the scaffold for plasminogen binding and activation by tPA. However, until recently, the plasminogen receptors that play a role in cell migration were not well defined. During inflammation, cellular migration is important because inflammatory cells must get to the desired tissue in order to resolve inflammation effectively.

Nuclear protein histone H2B (H2B) was identified as a prominent macrophage-binding plasminogen receptor in a peritoneal thioglycollate model. Fragment antigen binding (Fab) fragments for H2B and several other plasminogen-binding receptors including  $\alpha$ -enolase, annexin 2, and S100A10 (p11) can be used to block plasminogen binding on macrophages. Fab fragments directed against H2B have the greatest impact on



plasminogen binding to macrophages, but Fab fragments against the other receptors also have some effect on plasminogen binding. Mice treated intravenously with anti-H2B Fab had ~50% less macrophage recruitment in response to peritoneal thioglycollate without affecting circulating monocyte levels (Das et al., 2007). In addition, *S100A10*<sup>-/-</sup> mice had ~50% decreased macrophage migration into the peritoneal cavity when stimulated with thioglycollate, and *S100A10*-deficient macrophages show an eightfold decrease in migration through Matrigel (O'Connell et al., 2010). Interestingly, treatment with L-type Ca<sup>2+</sup> channel blockers, which block expression of plasminogen receptors on the surfaces of macrophages including H2B,  $\alpha$ -enolase, annexin 2, and *S100A10*, blocks recruitment of macrophages into the peritoneal cavity in response to thioglycollate (Das et al., 2009).

In 2010, a novel cell-surface plasminogen receptor, PLG-R<sub>KT</sub>, was discovered and found to be colocalized with uPA and uPAR on migratory cells that include leukocytes and neuronal cells. PLG-R<sub>KT</sub> enhances plasminogen activation and can also bind tPA to further enhance plasminogen activation (Andronicos et al., 2010). Macrophages from *Plg-R<sub>KT</sub>*<sup>-/-</sup> mice have a decreased ability to bind plasminogen (Miles et al., 2017), and in the intraperitoneal thioglycollate model, *Plg-R<sub>KT</sub>*<sup>-/-</sup> mice show ~80% reduced macrophage recruitment into the peritoneal cavity (Andronicos et al., 2010). In addition, PLG-R<sub>KT</sub> seems to modulate M2 macrophages specifically; in a pleurisy model, *Plg-R<sub>KT</sub>*<sup>-/-</sup> mice show impaired recruitment of mononuclear cells into the pleural cavity in response to LPS. They have an increase in M1 macrophages in the pleural cavity and no change in M2 macrophage levels. This suggests that there is an impairment in polarization of macrophages from an M1 proinflammatory phenotype to an M2 anti-inflammatory phenotype, which causes a defect in ability to resolve inflammation (Vago et al., 2019).

**Resolution of inflammation and wound healing.** It is crucial that inflammation is resolved in a timely manner after an insult to avoid chronic inflammation, which may have detrimental effects. Resolution is controlled by a decrease in chemokine concentration and inhibition of neutrophils in the injured tissue, and resolution pathways are activated shortly after the initial inflammatory response (Sugimoto et al., 2016). Plasminogen-deficient mice have impaired wound healing (Rømer et al., 1996; Vago et al., 2019). Plasminogen is transferred to wound sites by inflammatory cells early during healing (Shen et al., 2012), where it plays a role in clearing fibrin and neutrophils and forming new connective tissue and blood vessels during the wound healing process (Sulniute et al., 2016).

*Plg*<sup>-/-</sup> mice, as well as *Plg-R<sub>KT</sub>*<sup>-/-</sup> mice, show deficits in the ability to resolve inflammation due to a lack of ability to polarize M1 macrophages into M2 macrophages (Vago et al., 2019). Plasminogen induces macrophage reprogramming from M1 to M2 polarization in order to promote resolution of inflammation. In the resolution phase of LPS-induced inflammation, plasminogen and plasmin are up-regulated in order to polarize macrophages into anti-inflammatory subtypes. In addition, if plasminogen or plasmin is given when inflammation is peaking, this leads to increased neutrophil apoptosis and efferocytosis via

annexin A1 (Sugimoto et al., 2017). Furthermore,  $\alpha$ -enolase, a plasminogen receptor, is necessary for inflammation resolution, and inhibitors of  $\alpha$ -enolase-plasminogen binding lead to inadequate restoration of homeostasis to injured muscle tissue (Díaz-Ramos et al., 2012).

### Plasminogen plays a role in the development of many pathologies

Plasminogen is important for many different systems and organs in the body, and thus its regulation has implications for a wide spectrum of pathologies and diseases. Below, we review the different types of pathologies that plasminogen's inflammatory roles may regulate. Table 1 summarizes the roles of plasminogen, tPA, and uPA in models of disease described below and indicates whether there is evidence that the role in the disease model is fibrin dependent.

#### Infection

Plasminogen has been extensively studied in infectious inflammation. Plasminogen binding is crucial for optimal host invasion for certain bacteria, viruses, fungi, and parasites. These infectious agents use the proteolytic activity of plasminogen to get through tissues, proliferate, and produce an inflammatory response.

**Bacterial infection.** A large number of pathogens express receptors that can bind to plasminogen, immobilizing plasminogen on the bacterial cell surface where plasmin can then participate in proteolytic functions to help the bacteria spread throughout the body (Lähteenmäki et al., 2001; Bhattacharya et al., 2012).

LPS, a protein on the outer membrane of gram-negative bacteria, can elicit a strong inflammatory response. Plasminogen is up-regulated in the cerebral spinal fluid and urine of LPS-treated rats and originates in the blood (Mezzapesa et al., 2014). PAI-1 is also up-regulated in response to LPS, and knockdown of PAI-1 in rat macrophages leads to decreased inflammatory cytokine production when stimulated with LPS (Wang et al., 2014).

Many bacteria have their own receptors for plasminogen. For example, the gram-negative bacteria *Moraxella catarrhalis* and *Pseudomonas aeruginosa*, as well as spirocyclic bacteria *Borrelia crocidurae* and *Borrelia burgdorferi*, each bind plasminogen for host invasion (Singh et al., 2015; Ceremuga et al., 2014; Nordstrand et al., 2001; Gebbia et al., 1999; Coleman et al., 1995). In addition, gram-positive bacteria, such as *Streptococcus pneumoniae*, bind plasminogen and uPA to induce plasmin recruitment and initiation of bacterial invasion into the host while evading the normal innate immune response (Agarwal et al., 2013; Sanderson-Smith et al., 2013). *Staphylococcus aureus*, a gram-positive bacteria that causes infection of the skin and soft tissues, encodes its own plasminogen activator, staphylokinase, which induces plasmin activity and aids in invasiveness of *S. aureus*, inducing larger lesions in the host and decreased ability of the host to clear the bacteria (Peetermans et al., 2014). When mice were infected with *S. aureus*, *Plg*<sup>-/-</sup> or *tPA*<sup>-/-</sup>/*uPA*<sup>-/-</sup> mice showed the highest survival rate, WT and *tPA*<sup>+/-</sup>/*uPA*<sup>+/-</sup> mice showed the lowest survival rate, and *Plg*<sup>+/-</sup> and *tPA*<sup>-/-</sup>/*uPA*<sup>-/-</sup> mice had an intermediate survival rate (Guo et al., 2011).

Table 1. Summary of experiments indicating the role of plasminogen activator system in animal models of disease

Authors	Model	Plasmin(ogen) role	tPA role	uPA role	Fibrin dependent?
<b>Infection</b>					
Nordstrand et al., 2001	<i>Plg</i> <sup>-/-</sup> mice on C57Bl/6 background	Aids in infection by <i>B. crocidurae</i>			
Gebbia et al., 1999	<i>Plg</i> <sup>-/-</sup> mice on mixed 129/Black Swiss background	Increases bacterial load in inflammation in brains and hearts of mice treated with <i>B. burgdorferi</i>			
Coleman et al., 1995	Human umbilical vein endothelial cells	Aids in <i>B. burgdorferi</i> penetration of endothelium			
Guo et al., 2011	<i>Plg</i> <sup>-/-</sup> , <i>tPA</i> <sup>-/-</sup> , and <i>uPA</i> <sup>-/-</sup> mice on C57Bl/6 background	Aids in infection by <i>S. aureus</i>	Aids in infection by <i>S. aureus</i>	Aids in infection by <i>S. aureus</i>	
Berri et al., 2013	<i>Plg</i> <sup>-/-</sup> mice on C57Bl/6 background	Aids in lung inflammation in response to infection with H5N1 and H1N1 viruses			Yes
Stie and Fox, 2012a, 2012b	Primary cultured BMEC	Used by the fungus <i>C. neoformans</i> to invade its host		Upregulated in response to <i>C. neoformans</i> infection	
González-Miguel et al., 2019	Wistar rats	Enhanced generation in response to parasite <i>F. hepatica</i> infection			
Silva et al., 2019	<i>Plg</i> <sup>-/-</sup> and <i>Fbg</i> <sup>-/-</sup> mice on C57Bl/6 background	Necessary for macrophage migration in sterile peritonitis			Yes
<b>Neurological disease</b>					
Tsirka et al., 1997a, 1997b	C57Bl/6 mice	Regulates neuronal death in response to acute kainate injection	Regulates microglial activation and neuronal death in response to acute kainate injection		No
Hultman et al., 2014	<i>Plg</i> <sup>-/-</sup> mice on a C57Bl/6 background	Promotes neuroinflammation in response to acute LPS challenge			No
Baker et al., 2019	C57Bl/6 mice	Recruits perivascular macrophage migration and microglial activation in response to peripheral LPS challenge			
Paul et al., 2007	<i>Plg</i> <sup>-/-</sup> AD mice	Contributes to neurovascular damage in a mouse model of AD			Yes
Shaw et al., 2017	<i>Plg</i> <sup>-/-</sup> mice on a C57Bl/6 background	Promotes demyelination in EAE model			Yes
Baker et al., 2018	Tg6799 mice	Promotes neuroinflammation and AD pathology			
Siao and Tsirka, 2002	<i>tPA</i> <sup>-/-</sup> mice on a C57Bl/6 background		Promotes microglial activation in response to LPS		
East et al., 2005; Dahl et al., 2016	<i>tPA</i> <sup>-/-</sup> mice on a C57Bl/6 background		Protects against early onset and severe demyelination in EAE model		Yes
Lenglet et al., 2014	129/SvEV mice		Increases inflammation following stroke		No

Table 1. Summary of experiments indicating the role of plasminogen activator system in animal models of disease (Continued)

Authors	Model	Plasmin(ogen) role	tPA role	uPA role	Fibrin dependent?
<b>Arthritis</b>					
Li et al., 2005	<i>uPA</i> <sup>-/-</sup> and <i>Plg</i> <sup>-/-</sup> mice on a C57Bl/6 background	Promotes joint inflammation in systemic collagen type II-induced arthritis		Promotes joint inflammation in collagen type II-induced arthritis	
Raghu et al., 2014	<i>Plg</i> <sup>-/-</sup> mice on a C57Bl/6 background	Prevents joint inflammation in small joints and promotes inflammation in large joints in a monoarticular TNF- $\alpha$ arthritis model			Yes
Cook et al., 2010	<i>uPA</i> <sup>-/-</sup> mice on a C57Bl/6 background			Necessary for systemic arthritis development	
De Nardo et al., 2010	<i>uPA</i> <sup>-/-</sup> mice on a C57Bl/6 background			Limits susceptibility to monoarticular arthritis	Yes
<b>Other</b>					
Das et al., 2013	<i>Plg</i> <sup>-/-</sup> mice on a C57Bl/6 background	Promotes atherosclerosis progression via regulation of foam cell formation			
DeFilippis et al., 2016	Humans with myocardial infarction	Binds to OxPLs to regulate inflammation in myocardial infarction events			Yes
Swaisgood et al., 2007	<i>Plg</i> <sup>-/-</sup> mice on a C57Bl/6 background	Promotes mucus production and immune cell recruitment into lungs of ovalbumin-induced asthma model			
Li et al., 2011	BALB/c mice	Plasminogen is decreased and plasmin is increased in psoriatic lesions, promoting upregulation of chemokines			

Note that fibrinogen dependence is indicated only if it was specifically studied in the particular experiment described. BMEC, brain microvascular endothelial cells.

**Viral infection.** Influenza A viruses are associated with inflammation of the lungs. Plasminogen-deficient mice showed decreased lung inflammation in response to infection with H5N1 and H1N1, two influenza A viruses, and this was dependent on fibrinolysis, as pharmacological depletion of fibrinogen reverses this reduction in lung inflammation (Berrì et al., 2013).

**Fungal infection.** The fungus *Cryptococcus neoformans* uses plasmin to degrade microvascular endothelial cells to invade the blood-brain barrier (BBB) in its host (Stie and Fox, 2012a). This fungus up-regulates urokinase expression as a mechanism by which plasmin can aid the fungus in endothelial cell invasion (Stie and Fox, 2012b). Plasminogen also binds directly to *Aspergillus fumigatus*, a common lung infection that affects immunocompromised people. Furthermore, genetic variation in the plasminogen allele is correlated with risk of human development of this infection following hematopoietic stem cell transplant (Zaas et al., 2008).

**Parasitic infection.** *Fasciola hepatica* is a type of parasitic trematode that infects the livers of humans and other mammals through food. Interestingly, humans infected with *F. hepatica* have some neurological issues. A proteomic analysis of *F. hepatica* identified several new plasminogen-binding proteins that lead to enhanced plasmin generation, contributing to BBB damage (González-Miguel et al., 2019).

### Neuroinflammatory disease

**Plasminogen activator system in the central nervous system (CNS).** While the liver is the primary source of plasminogen in the body, neuroendocrine tissues, as well as neurons in the cortex, hippocampus, and cerebellum, express plasminogen (Mehra et al., 2016). In the CNS, plasminogen activation is primarily dependent on regulation by tPA and PAI-1. The major source of tPA in the brain is endothelial cells of microvessels (Levin et al., 1997). However, tPA can be expressed by a variety of cells in the CNS, including neurons, microglia, astrocytes, oligodendrocytes, perivascular mast cells, pericytes, and infiltrating leukocytes, suggesting that tPA may have a variety of roles within the brain (Yepes et al., 2009; Mehra et al., 2016), including neuronal migration, synaptic outgrowth, neurotransmission, and synaptic plasticity (Huang et al., 1996; Madani et al., 1999; Zhuo et al., 2000). tPA protein and mRNA are localized at the synapse of neurons, and when released into the extracellular space, tPA can act on plasminogen and can be inhibited by PAI-1 (Qian et al., 1993). uPA is normally expressed at low levels in the brain by some neurons and astrocytes, but can be up-regulated in some pathological conditions including multiple sclerosis (MS) and epilepsy (Mehra et al., 2016). In addition to PAI-1, neuroserpin is a tPA and uPA inhibitor that is

expressed by neurons of the CNS. Neuroserpin forms unstable complexes with tPA, dissociating quickly, indicating that it is a transient inhibitor in the nervous system (Osterwalder et al., 1998).

**Microglia and innate brain inflammation.** Microglia are the specialized resident macrophages of the brain that mediate the neuroimmune response by releasing cytokines and signaling molecules to clear cellular debris and by phagocytosing dead neurons. Many neurological diseases, especially neurodegenerative diseases such as Alzheimer's disease (AD), MS, and stroke, are associated with activation of the glial cells of the brain that can induce neuronal death and harm the brain.

tPA can regulate microglial activation in response to excitotoxin-mediated neurodegeneration, independent of plasminogen activation (Tsirka et al., 1997b). Furthermore, plasminogen-deficient mice are resistant to neurodegeneration induced by acute kainate injection (Tsirka et al., 1997b; Tsirka et al., 1997a), and this function is independent of fibrin (Tsirka et al., 1997a). Plasminogen and plasmin both induce microglial activation and expression of IL-1 $\beta$ , TNF- $\alpha$ , and reactive oxygen species (Min et al., 2003). When blood-derived plasminogen is knocked down at the mRNA level, microglial and astrocytic activation are decreased significantly following injection with LPS, possibly due to less migration of perivascular macrophages into the brain during LPS challenge (Baker et al., 2019). Fibrinogen can also induce microglial activation through its  $\alpha_M\beta_2$ -binding motif, and blocking fibrinogen- $\alpha_M\beta_2$  interactions reduces proinflammatory microglial activation (Adams et al., 2007).

In addition, tPA enhances recruitment of microglia following stroke in a mouse model. Recombinant human tPA (rtPA) is often used following acute ischemic stroke as the standard of care. However, rtPA increases inflammation significantly when administered to mice following stroke by up-regulating chemokines, cytokines, and microglial recruitment, leading to increased mortality in mice treated with rtPA (Lenglet et al., 2014). tPA can promote microglial activation independent of its proteolytic functions by binding to annexin II and galectin 1 on microglia (Siao and Tsirka, 2002).

**MS.** MS is a progressive demyelinating disease of the brain and spinal cord that leads to issues with muscle control and basic body functions. MS patients have altered expression of tPA and PAI-1 in white matter regions of the brain and spinal cord (Cuzner et al., 1996) and increased PAI-1 levels in the plasma (Onodera et al., 1999). A commonly used mouse model of MS is experimental autoimmune encephalomyelitis (EAE), whereby an antigen is injected that induces progressive demyelination.

Plasminogen deficiency protects from demyelination in an EAE model. In this mouse model, *Plg*<sup>-/-</sup> mice with induced EAE have delayed disease onset and reduced disease severity compared with WT animals, corresponding to much less macrophage/microglial accumulation in the spinal cords, as well as decreased demyelination and paralysis. Loss of plasminogen impeded macrophage migration during disease progression, leading to decreased neuroinflammation that was dependent on plasmin-mediated fibrinolysis, as supported by data from *Fbg*<sup>-/-</sup> and *Plg*<sup>-/-</sup>/*Fbg*<sup>-/-</sup> mice. Both *Fbg*<sup>-/-</sup> and *Plg*<sup>-/-</sup>/*Fbg*<sup>-/-</sup> mice have a decreased severity of EAE, but no delay in disease onset,

suggesting that plasmin-mediated fibrinolysis is a key mediator of neuroinflammation in the spinal cords in this EAE model (Shaw et al., 2017).

tPA is implicated in MS development and is up-regulated fourfold when EAE is induced. It seems to play a protective role in EAE, as tPA-deficient mice have an earlier onset and increased severity of demyelination (East et al., 2005; Dahl et al., 2016), likely because tPA is necessary to clear fibrin deposits on axons damaged during EAE progression. Axonal damage is a crucial contributor to MS progression, and fibrinogen has been shown to be a critical regulator of microglial activation and axonal damage in EAE (Davalos and Akassoglou, 2012).

**AD.** There is conflicting evidence about the role plasminogen may play in the pathology of AD. On one hand, plasmin mediates a proinflammatory response. On the other, plasmin participates in fibrinolysis to break down fibrin, which itself is proinflammatory. In addition, there is some evidence that plasmin can degrade  $\beta$ -amyloid (A $\beta$ ), a protein that aggregates in the parenchyma of AD patient brains and is also a proinflammatory protein (Tucker et al., 2000; Saïdo and Leissring, 2012). In human brain tissue from AD patients, tPA, uPA, PAI-1, and  $\alpha_2$ -antiplasmin levels are increased compared with those of age-matched controls, suggesting that the plasminogen activator system may be up-regulated in order to try to clear A $\beta$  during the disease process (Barker et al., 2012).

Fibrin has been implicated in the pathogenesis of AD (Cortes-Canteli et al., 2010; Cortes-Canteli et al., 2012). Accumulation of fibrin in the vasculature of the AD brain leads to neurovascular damage and inflammation in the AD brain. AD patient brains seem to have reduced tPA activity, plasmin levels, and fibrin clearance (Ledesma et al., 2000; Melchor et al., 2003). AD mice heterozygous for a plasminogen-knockout allele (*Plg*<sup>+/-</sup>) have increased neurovascular damage compared with AD mice WT for plasminogen, and AD mice heterozygous for a fibrinogen  $\alpha$ -chain knockout allele (*Fbg*<sup>+/-</sup>) have decreased BBB damage. In addition, pharmacological clearance of fibrin using the protease anicrod reduces a neuroinflammatory response and microvascular injury, whereas plasmin inhibition using tranexamic acid exacerbates neuroinflammation and neurovascular damage in an AD mouse model (Paul et al., 2007).

However, in some cases, although plasmin deficiency causes increased fibrin accumulation, this does not necessarily lead to an increased immune response, indicating the important role that plasminogen plays in inflammation independent of fibrinolysis. *Plg*<sup>-/-</sup> mice injected intrahippocampally with LPS show increased parenchymal fibrin deposits but a decreased neuroinflammatory response to LPS, suggesting that plasmin may be important to a normal neuroinflammatory response, at least in response to an acute inflammatory challenge (Hultman et al., 2014). In addition, plasmin may help drive the neuroinflammatory response in AD, as knockdown of blood plasminogen using an antisense oligonucleotide directed against liver-produced plasminogen leads to decreased activation of microglia and astrocytes, decreased migration of perivascular macrophages into the brain, and decreased AD pathology in a mouse model of AD (Baker et al., 2018). It is likely that plasminogen may play different roles in AD progression depending on the stage of disease and degree of BBB damage.



## Arthritis

The plasminogen activator system is differentially regulated in rheumatoid arthritis, and rheumatoid arthritis patients have increased uPA, uPAR, and PAI-1 levels, as well as decreased tPA levels in their synovial tissues (Busso et al., 1997). In addition, in a mouse model of systemic arthritis in which joint inflammation is induced by immunizing mice with collagen type II, uPA and plasminogen both play a role in development of inflammation. uPA-deficient mice have a reduced severity of arthritis in the paws, and while ~80% of WT mice develop arthritis following collagen type II injection, no plasminogen-deficient animals develop arthritis within 40 d of injection. When the plasminogen-deficient animals are supplemented with exogenous plasminogen, mice develop arthritis and inflammatory cells migrate into the joints within 5 d (Li et al., 2005). It is suggested that plasminogen may thus be important to the early development of collagen-induced arthritis (Judex and Mueller, 2005).

Plasminogen may play both a proinflammatory and an anti-inflammatory role in arthritis development depending on the model used and the location of the joint inflammation. TNF- $\alpha$ -induced arthritis is a monoarticular model in which arthritis only affects a specific joint. Plasminogen deficiency exacerbates TNF- $\alpha$ -induced arthritis severity in the joints of the paws in mice. However, in the larger joints of the knee of these same mice, plasminogen deficiency has the opposite effect, decreasing arthritis severity. This effect was dependent on fibrinogen, and genetic depletion of fibrinogen reversed the proinflammatory and anti-inflammatory effects of plasminogen deficiency in the paw joints and knee joints, respectively. In addition, MMP-9 seems to be a driving factor for plasminogen's different effects on various joints, as MMP-9 was reduced in the knee joints of plasminogen-deficient mice but not in the paw joints. Taken together, these data point to two separate roles of plasminogen and fibrinogen that depend on the local environment of the arthritic joint (Raghu et al., 2014).

Since the plasminogen activator system seems to have a differential effect on arthritis progression depending on the type of arthritis model (systemic or monoarticular), it is important to understand the underlying mechanistic differences driving this difference. In the systemic arthritis model using collagen type II immunization, uPA originating from the bone marrow is required for full arthritis development (Cook et al., 2010). However, if uPA-deficient mice also have an acute injection into a joint, this increases their susceptibility to monoarticular arthritis and inflammation (De Nardo et al., 2010). Thus when uPA is required for immune complex formation and complement system activation, it can be detrimental to arthritis development, whereas in monoarticular models, where arthritis is induced by local trauma and wound healing is a necessary process, uPA may be protective via a plasmin-mediated fibrinolysis mechanism (Cook et al., 2010, De Nardo et al., 2010).

## Cardiovascular disease

Plasminogen has been implicated in cardiovascular diseases including atherosclerosis, thrombotic events, and myocardial infarction. Inflammation and plasminogen receptors are important for proper functional leukocyte responses in cardiovascular health.

Plasminogen receptor expression is regulated by Ca<sup>2+</sup> channels, and this interaction has been implicated in cardiovascular pathology and inflammation (Das et al., 2010).

Foam cells are a type of macrophage that localize to fatty atherosclerotic deposits on blood vessel walls where they ingest lipids. The formation of foam cells is important to the development and progression of atherosclerosis. Plasminogen regulates other macrophage and monocyte-derived cell types, and this extends to the regulation of foam cell formation via macrophage expression of CD36 and leukotriene B<sub>4</sub>. Thus, plasminogen may play a role in atherosclerosis progression (Das et al., 2013). In addition, uPA and uPAR are up-regulated in atherosclerotic lesions in diseased arteries, and levels correlate with disease severity (Kienast et al., 1998; Steins et al., 2004).

With aging, changes in coagulation and fibrinolysis are associated with an increased risk of thrombotic events caused by vessel occlusion. These clots can come loose and clog a vessel in the body to cause pulmonary embolism, stroke, or issues in the kidneys, legs, or gastrointestinal tract. A genetic variant in uPAR (PLAUR rs344782) was identified in the Cardiovascular Health Study as a protective factor against venous thromboembolic disease (Reiner et al., 2009). Soluble uPAR has also been indicated as a risk factor for venous thromboembolic disease in another cohort of patients from the Malmo Diet and Cancer study in Sweden (Engström et al., 2016).

Plasminogen is also associated with acute myocardial infarction (AMI). Proinflammatory oxidized phospholipids (OxPLs) bind to plasminogen covalently and are increased in patients following AMI. These OxPLs serve to shorten lysis time for fibrin clots, and OxPL-PLG levels return to baseline in the months following AMI (Leibundgut et al., 2012). OxPL-PLG levels differ depending on whether AMI is an atherothrombotic (type 1) or nonatherothrombotic (type 2) event. In type I patients, both plasminogen and OxPL-PLG are significantly lower than in type 2 patients, supporting the idea that OxPL-PLG is important for fibrinolysis in certain types of AMI (DeFilippis et al., 2016). It may also be true that lower levels of plasminogen and OxPL-PLG are risk factors for worse outcomes following AMI, although this has not been studied in detail and warrants further investigation.

## Asthma

Asthma is a condition in which there is significant inflammation of the airways, leading to breathing difficulty, and there is evidence that the plasminogen system plays a role in the development of asthma pathology. In a mouse model in which asthma is induced using ovalbumin, there is a significant decrease in eosinophils and lymphocytes, as well as in mucus production in the lungs of plasminogen-deficient mice. Furthermore, mice treated with the plasmin inhibitor tranexamic acid show a similar decrease as *Plg*<sup>+/-</sup> and *Plg*<sup>-/-</sup> mice (Swaisgood et al., 2007).

PAI-1 is also implicated in asthma pathogenesis. PAI-1 levels are up-regulated in the plasma of human asthma patients (Cho et al., 2011). In addition, ovalbumin-challenged mice that have been treated with tiplaxtinin, a PAI-1 inhibitor, show decreased inflammatory cell migration into the lungs and a decrease in airway collagen deposition (Lee et al., 2012).

### Psoriasis

Psoriasis is a chronic inflammatory skin disorder characterized by a dermal infiltrate made of T cells, dendritic cells, and macrophages (Li et al., 2011), and plasminogen activators are elevated in the skin lesions of psoriasis patients (Gissler et al., 1993). In addition, plasminogen is diminished in these lesions, accompanied by an increase in plasmin activity and an increase in annexin II, a plasmin receptor. This plasmin increase is associated with an up-regulation of chemokines and other inflammatory factors in these lesions (Li et al., 2011).

### Conclusions

Plasminogen, its activators, and its receptors comprise pathways that play roles in various inflammation regulatory processes. These roles span functions in fibrinolysis, interaction with complement proteins, ECM degradation, inflammatory cell migration, and resolution of inflammation and wound healing. As a consequence, plasminogen has been associated with many pathologies spanning different organ systems, including the blood (infection, systemic inflammation, complement activation, and cardiovascular disease), the brain (neuroinflammation and neurodegenerative disease), the joints (arthritis), the lungs (asthma), and the skin (psoriasis). In addition, the plasminogen activator system is found to be differentially regulated in plasma or inflammatory lesions in a variety of these diseases.

A summary of these diseases, along with the known effect of tPA or uPA modulation, is given in Table 1. Fibrinogen dependence was not assessed in all studies, limiting our ability to make definitive conclusions about the role of plasminogen-dependent fibrinolysis in mediating inflammatory processes. However, in models of peripheral disease, such as infection, arthritis, and AMI, fibrinogen dependence was found to be a contributor to plasminogen's inflammatory functions. Studies using different models of arthritis give further insight into fibrin dependence of plasminogen-mediated processes. In two different models of arthritis, systemic and acute, fibrin dependence was only seen when arthritis was acutely induced. Thus, in acute processes, when fibrinolysis is necessary for wound healing, plasminogen may be protective against chronic inflammation. However, during chronic inflammatory insults and diseases, plasminogen may be detrimental because it allows for persistent migration of inflammatory cells and activation of inflammatory modulators.

In the CNS, fibrin independence and fibrin dependence are both reported for plasminogen's roles in neuroinflammatory disease. These results may be confounded by the fact that plasminogen and its activators are produced endogenously in the brain, separate from liver-produced plasminogen. Conditional or tissue-specific knockouts of plasminogen and its activators may be necessary to further understand the specific roles of brain-plasminogen in neurological disease.

One common link between diseases across organ systems is that plasminogen seems to play a role in development of pathology in several autoimmune models. For example, in arthritis models where collagen immunization is used to induce joint inflammation, plasminogen is necessary for cell migration into the joints. In models of neurodegenerative disease, such as EAE where myelin immunization is used to model MS or AD mouse

models in which the A $\beta$  protein is overexpressed in the brain, plasminogen is also necessary for full inflammatory cell migration and responses in the nervous system, contributing to the chronic inflammation associated with neurodegenerative disease.

Plasminogen and related proteins may serve as targets for a wide variety of pathologies, all connected to their role in inflammatory regulation. However, the pleotropic functions of plasminogen also serve as a limitation to its use as a therapeutic target unless highly specific therapeutics are found that could alter one function of plasminogen without affecting another. Humans with plasminogen deficiency have well-characterized pathologies related to deposition of fibrous deposits on mucosal membranes throughout the body, limiting plasminogen's potential as a therapeutic target itself.

Future work in the field of plasminogen and inflammation should focus on better defining the pathways that lead to inflammatory effects. Several receptors for plasminogen have been discovered that contribute to its inflammatory roles, such as PLG-R<sub>KT</sub>. Attention to these receptors and their unique or combined roles in inflammatory processes may yield important insights into the inflammatory diseases in which plasminogen plays a role. Specific modulation of these receptors has the potential to bypass the detrimental effects that targeting plasminogen itself would have on other functions within the body and warrants future study.

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