JLB

Review

Plasmin as a proinflammatory cell activator

Tatiana Syrovets, Oleg Lunov, and Thomas Simmet[1]

Institute of Pharmacology of Natural Products and Clinical Pharmacology, Ulm University, Ulm, Germany

RECEIVED FEBRUARY 2, 2012; REVISED APRIL 7, 2012; ACCEPTED APRIL 19, 2012. DOI: 10.1189/jlb.0212056

ABSTRACT

The serine protease plasmin generated from its zymogen plasminogen is best known for its function as a key enzyme of the fibrinolytic cascade. However, beyond fibrinolysis, plasmin has a number of crucial functions in a variety of processes, including inflammation. Various cells can bind plasminogen and plasmin via plasminogen-binding sites exposing a C-terminal lysine. Plasmin, generated as a result of plasminogen activation at the cell surface, is protected from its physiological inhibitors. Apart from its ability to facilitate cell migration in tissues, plasmin is capable of triggering signaling, which depends on cellular binding via its lysine-binding sites and its proteolytic activity. Plasmin-induced signaling affects various functions of monocytes, macrophages, DCs, and others, with the list of affected cells still growing. In vitro and in vivo studies have demonstrated the ability of plasmin to stimulate the production of cytokines, ROS, and other mediators, thereby contributing to inflammation. Plasmin-induced chemotaxis of monocytes and DCs indicates that it is also a potent chemoattractant for immune cells. Therefore, excessive activation of plasmin in chronic inflammatory or autoimmune diseases might exacerbate the activation of inflammatory cells and the pathogenesis of the disease. This review focuses on the available evidence for physiological and pathophysiological roles the serine protease plasmin in inflammatory processes. *J. Leukoc. Biol.* 92: 509–519; 2012.

Introduction

The serine protease plasmin represents the key enzyme of the fibrinolytic cascade and is generated by proteolytic cleavage of its precursor plasminogen, which is synthesized by liver cells as a 810 aa glycoprotein. After cleavage of a 19-aa-long signaling peptide, plasminogen is released into plasma, where its concentrations reach 2.4 μM [1]. Under physiological or pathological conditions, single-chain plasminogen is converted to the two-chain plasmin molecule by cleavage between Arg561 and Val562. Both, plasmin as well as the plasminogen molecule, contain five homologous kringle domains (**Fig. 1**). Crystallographic structures are currently available of only the four kringles, as well as of the B-chain of plasminogen or plasmin, with the active site containing 3 aa—His603, Asp646, and Ser741 [3–8] (Fig. 1). Both molecules bind via those kringle structures to terminal lysine on substrates, inhibitors, and cell surface-binding sites [1]. Although the kringle structures are highly homologous, it is Kringle 1, followed by Kringles 4 and 5 that have the highest lysine-binding affinity. Kringles 1–3 bind to platelets and fibrinogen, whereas Kringle 4 does not [1, 9, 10]. Proteolytic processing of plasminogen leads to exposure of its active catalytic center, reflecting transition of the zymogen plasminogen into the active serine protease plasmin.

Plasmin generation

Physiologic plasminogen activators are tPA and uPA, two serine proteases with high specificity toward plasminogen. tPA is secreted by endothelial cells and exhibits higher proteolytic activity when bound to cells or fibrin [11]. uPA is likewise produced by endothelial cells but also by monocytes and macrophages. Cell surface-bound uPA converts plasminogen to plasmin much more efficiently than in solution [12]. Plasmin, in turn, cleaves and converts tPA and uPA into two-chain proteases, which exhibit higher proteolytic activity, implying a positive feedback on the fibrinolytic cascade [11]. In this context, uPA, expressed by monocytes or endothelial cells, is believed to be the major physiological activator of plasminogen bound to biological surfaces via lysine-binding sites [13].

Kallikrein, a serine protease activated in the course of contact activation, is another physiological activator of plasmin [14]. Contact activation takes part in a range of pathological conditions, including cardiovascular and renal disorders, inflammation, diabetes, asthma, and possibly cancer. Contact of plasma with negatively charged surfaces, such as proteoglycans, endotoxic LPS, or different types of crystals, triggers "contact" activation of coagulation factor XII, which, in turn, activates prekallikrein to kallikrein [14]. The components of the contact activation cascade could also be assembled at the surface of various cell, such as leukocytes, platelets, or endothelial cells, initiating the kallikrein activation, as well as the kal-

Abbreviations: BDNF=brain-derived neurotrophic factor, CTA=Commitee on Thrombolytic Agents, E-cadherin=epithelial cell cadherin, MMP=matrix metalloproteinase, PAR=protease-activated receptor, Plg-R_{KT}=plasminogen receptor, plasminogen receptor KT, ROS=radical oxygen species, tPA=tissue plasminogen activator, uPAR=urokinase-type plasminogen activator

1. Correspondence: Institute of Pharmacology of Natural Products and Clinical Pharmacology, Ulm University, Helmholtzstr. 20, D-89081, Ulm, Germany. E-mail: thomas.simmet@uni-ulm.de

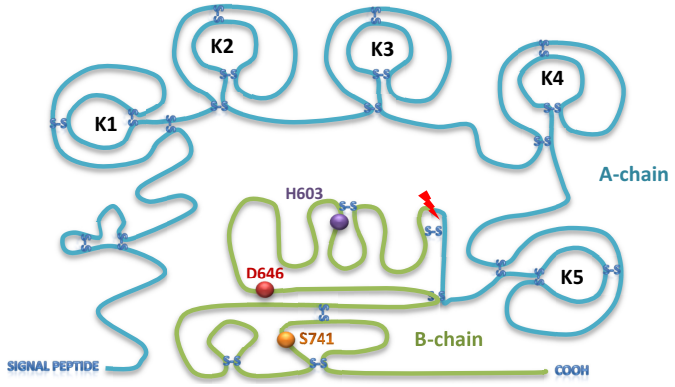

Figure 1. Schematic presentation of the plasminogen structure. The cleavage site converting plasminogen into the two-chain plasmin molecule is marked with a red arrow; amino acids of the catalytic site, H603, D646, and S741 are shown. K, Kringles; modified according to ref. [2].

likrein-mediated activation of plasminogen [15]. Thus, contact activation, which takes place during inflammation and other pathological conditions, might result in the generation of plasmin by kallikrein [14].

Regulation of plasmin activity

The major physiological inhibitor of plasmin is α_2-antiplasmin. It is present in plasma in concentrations high enough to almost immediately neutralize up to 1.2 μM unbound plasmin. Further, 2.4 μM free plasmin could be neutralized by plasma α_2-macroglobulin [16]. As a result of rapid inactivation of plasmin by its inhibitors, plasma levels of plasmin-α_2-antiplasmin complexes serve as a measure of plasmin generation and fibrinolytic activity [17]. However, plasmin synthesized or located on biological surfaces is protected from the inhibition by its physiological inhibitors [12]. Simultaneous binding of plasminogen and its activators to the cell surface concomitantly increases the rate of plasmin generation by its activators and protects plasmin from inactivation by α_2-antiplasmin, leading to enhanced pericellular plasmin concentrations on cells expressing plasminogen activators and plasminogen receptors. This makes the pericellular plasmin generation an important physiological or pathological factor influencing the cellular microenvironment.

Recently, plasmin generation has also been demonstrated on endothelial cell-derived microparticles [18]. Microparticles exhibiting a higher surface area to volume ratio compared with that of cells represent a relatively larger surface for the assembly of plasminogen activator/plasminogen complex and can thus promote local plasmin generation. TNF-α stimulates formation of endothelial cell-derived microparticles, which express uPA as well as uPAR on their surface. Such microparticles bind plasminogen in a lysine-dependent manner and support efficient generation of plasmin, which in turn, influences tube formation by endothelial progenitor cells with low amounts promoting tube formation and high amount having an inhibitory effect [18].

Plasmin substrates

Plasmin is an endopeptidase that hydrolyzes peptide bonds following arginine or lysine, similar to trypsin, although with a higher specificity for lysine [19]. High-efficacy cleavage of insoluble fibrin molecules by plasmin denotes the central role of plasmin in fibrinolysis. In addition to fibrin, plasmin cleaves coagulation factors V and VIII, several hormones (adrenocorticotropic and growth hormone, glucagon), metalloproteinases, growth factors, and matrix proteins [17]. Likewise, plasmin effectively cleaves complement factors C3 and C5, thereby releasing the respective chemotactic anaphylatoxin fragments [20]. Nonfibrin substrates of plasmin include the precursor of BDNF, proBDNF, produced mainly by neurons. Interestingly, it was found recently that proBDNF induces neuronal apoptosis, whereas cleaved BDNF elicits, as growth factor, the opposite, namely, growth-promoting effects on neurons. BDNF levels are abnormally reduced in manic and depressed states in bipolar disorders, as well as in long-term depression [21]. In addition, plasmin catalyzes cleavage of the carboxyl-terminal heparin-binding domain of VEGF, VEGF165, resulting in loss of its biological activity [22]. Plasmin can also cleave and decrease the biological activity of thrombopoietin, a hormone that regulates the production and differentiation of megakaryocytes, the precursors of platelets. Further, plasmin increases the local concentration of active TGF-β, which negatively regulates erythropoiesis and myelopoiesis [23]. Along with other proteases, plasmin cleaves the E-cadherin, leading to disruption of adherent junctions and release of a fragment of E-cadherin, soluble E-cadherin. Whereas, cell-bound E-cadherin is a well-documented tumor suppressor, soluble E-cadherin can promote cancer cell survival, migration, and invasion. Enhanced levels of soluble E-cadherin have been detected in fluids of tumor patients, in patients with viral and bacterial infections, and during organ failure [24]. Plasmin also induces post-translational modifications of the major human neutrophil attractant, IL-8 or CXCL8, cleaving it to 69–72 aa forms, which are highly potent neutrophil attractants in vivo [25].

PLASMIN-INDUCED CELL ACTIVATION

Plasminogen binds to and can be activated on the surface of various cell types through its lysine-binding sites [12]. Many pericellular effects of plasmin are mediated by matrix proteins or other mediators trapped by matrix and released proteolytically by plasmin [23, 26]. Initially, in vitro studies with distinct cells and protein components, as well as later experiments with genetically modified cells and animals, and the use of more specific inhibitors and antibodies have demonstrated that plasmin has profound, direct effects on monocytes and macrophages, endothelial and epithelial cells, as well as platelets [26], with the list of affected cells still growing (see Table 1).

Considering the role of plasmin in pericellular proteolysis and fibrinolysis, many cells express proteins on their surface, which may bind plasminogen/plasmin but do not elicit any intracellular signaling. Comprehensive reviews about plasmin/

ogen-binding sites and receptors reflecting different views have been published [12, 27, 28]. More recent studies highlighted a novel plasminogen receptor, called Plg-R_{KT}, which carries a C-terminal lysine, resides also on macrophages, and colocalizes with uPA receptors [29, 30]. Generally, most of the authors agree that the plasmin-induced intracellular signaling involves binding to terminal lysines via kringle structures and a proteolytic cleavage of a putative receptor, possibly similar to what has been demonstrated for thrombin-activated PARs [31, 32]. Interestingly, plasmin can, indeed, activate the PAR4 receptor expressed on platelets and induce platelet aggregation, although rather high concentrations of plasmin are necessary to activate human platelets [33, 34].

The signaling pathways induced in various cells by plasmin are remarkably similar. Activation of prosurvival PI3K/Akt and ERK1/2 signaling, of the chemotaxis-relevant Rho kinase pathway, or of transcription factors NF-κB and STAT families have been reported, which are all critically involved in the expression of many proinflammatory mediators [35]. Many studies include elaborated set-ups to prove that the cellular effects are, indeed, mediated by plasmin-induced proteolytic cleavage of a putative plasmin receptor. Still, each cell type seems to respond to plasmin stimulation quite differently.

Professional APCs

Monocytes, macrophages, and DCs serve as APCs, as well as direct effector and cytokine-producing cells. Accordingly, they play a central role in immune and inflammatory processes [36]. In response to various chemoattractants, they accumulate at the sites of inflammation as a result of directed migration, i.e., chemotaxis. In chronic inflammation, in arthritis, and in arteriosclerosis-associated inflammatory vascular injury, APCs play a predominant pathophysiological role [37, 38].

Monocytes and monocytic cells carry binding sites for plasminogen and uPA and can support efficient generation of plasmin on their surface [26]. We have studied extensively the effects of plasmin on monocytes and provided compelling evidence for the role of plasmin as a potent and specific activator of human monocytes [26, 39–48]. Plasmin generated as a result of contact activation of the intrinsic coagulation cascade or when added to cells kept in culture triggers biosynthesis of cysteinyl-leukotrienes and leukotriene B_4 by monocytes via the 5-lipoxygenase pathway. The effect is specific for plasmin, as a number of other proteases, including coagulation factors and other serine proteases, are unable to trigger a selective activation of the 5-lipoxygenase pathway in monocytes [44, 46, 48]. Plasmin binding to the monocyte surface occurs via kringle structures and lysine-binding sites, and the cell activation is dependent on the proteolytic activity of plasmin [48].

Moreover, plasmin elicits a PKC and cGMP-dependent chemotaxis and actin polymerization in monocytes [43] and stimulates a NF-κB-, AP-1-, and STAT-dependent expression of proinflammatory cytokines, including TNF-α, IL-1, and MCP-1, as well as tissue factor [41, 49]. The plasmin-induced chemotaxis, leukotriene release, and gene expression are all dependent on plasmin binding via the lysine-binding sites and on its proteolytic activity. In fact, binding of plasmin to monocytes and the induction of intracellular signaling are associated with proteolytic cleavage and dissociation of the annexin A2 heterotetramer, a complex found on the surface of monocytes, macrophages, and endothelial cells [50]. Which part of the truncated receptor is finally responsible for the initiation of intracellular signaling is still under investigation. The plasmin-induced chemotaxis is, however, not dependent on the activation of PAR1 or integrins α_1, α_V, α_L, α_M, β_1, β_2, β_3, and $\alpha_V\beta_3$, all implicated in chemotactic response and "possible" signaling partners for the plasmin receptor [50, 51].

Further, plasmin induces COX-2 and PGE_2, as well as MMP-1 production in monocytes, the latter clearly dependent on the annexin A2 heterotetramer and MAPK signaling. Accordingly, plasmin-induced MMP-1 expression is inhibited by neutralizing antibodies against the annexin A2 heterotetramer. Although bound to the monocyte plasminogen/plasmin-binding site, inactive plasmin fails to mimic the effects of the CTA plasmin [52]. Consistently, inactivated plasmin even acts as a competitive antagonist, inhibiting the effect of active plasmin on monocytes [43].

The half-life of monocytes in the circulation is rather short, only ~3 days. Blood monocytes act as a pool of progenitors, continuously repopulating tissue macrophages and DCs [53]. As tissue macrophages and DCs perform many physiological and pathological functions aimed to maintain tissue homeostasis or to exacerbate an inflammatory response, elimination of activated monocytes by apoptosis is of clinical relevance. On monocytic cells undergoing apoptosis, plasminogen binding is increased markedly [12]. Proteolytically active plasmin generated on the monocyte surface prevents apoptosis of monocytes induced by proinflammatory agents. This effect is dependent on PAR1 and is inhibited by an anti-PAR1 antibody [54].

Similar to monocytes, macrophages express the annexin A2 heterotetramer on their surface, which is likewise cleaved by plasmin, and plasmin induces activation of proinflammatory signaling in macrophages, e.g., activation of MAPKs, Akt, and transcription factors NF-κB and STAT, leading to release of the proinflammatory cytokines TNF-α and IL-6 [55]. Interestingly, macrophage migration to tumors depends on the expression of a S100A10, a component of the annexin A2 heterotetramer complex [56]. In this context, S100A10 seems to tether plasmin to the macrophage membrane, which will permit digestion of extracellular proteins and subsequent tissue penetration. Migration of macrophages into inflamed peritoneum apparently also depends on a plasmin receptor. Similarly, antibodies against Plg-R_{KT}, a membrane protein with a C-terminal lysine, which effectively binds plasminogen, block, at least partially, the macrophage migration to the peritoneum [30]. In this setting, extracellular plasmin is apparently essential for MMP activation and cleavage of different ECMs during in vivo migration and invasion [30].

Several receptors have been implicated in the mediation of plasmin signaling in monocytes and macrophages. The annexin A2 heterotetramer and Plg-R_{KT} have already been mentioned. Several interesting reports indicate that the annexin A2 heterotetramer may use a TLR to elicit intracellular signaling. Indeed, the signaling induced by the TLR4 ligand, endotoxic LPS, is similar to that elicited by plasmin, encompassing activation of Akt, MAPK, and NF-κB, followed by release of a

number of proinflammatory mediators [41, 50, 57, 58]. Soluble annexin A2 heterotetramer activates in human macrophages MAPK and NF-κB and induces release of TNF-α, IL-6, IL-1α and several chemokines, including MCP-1. This signaling is abrogated in macrophages lacking TLR4, suggesting that annexin A2 cooperates with TLR4 to activate macrophages [59, 60]. In the RAW264.7 macrophage cell line, plasmin activates the NF-κB-dependent IL-8 promoter, as well as TNF-α release and potentiates the LPS-induced activation of NF-κB and TNF-α release. These effects critically depend on the proteolytic activity of plasmin and occur independent from PARs [61].

DCs are the most potent, professional APCs, strategically located at the sites of antigen entry. Upon optimal maturation and activation, DCs produce IL-12 and other cytokines and are able to define immune responses by directing the T cell differentiation into different T cell subsets. In this respect, soluble as well as membrane-bound signals, delivered among DCs and T cells, are essential for the initiation of the immune response [62]. We investigated whether plasmin might alter the function of human DCs. Plasmin elicits a time-dependent actin polymerization and chemotaxis in DCs. The plasmin-induced chemotaxis is dependent on activation of Akt and ERK1/2 kinases, which induce the phosphorylation and activation of the chemotaxis-relevant regulatory myosin light chain. DCs express Akt1 and Akt2; however, unexpectedly, plasmin activates exclusively Akt2. Knockdown of Akt2 in DCs with short hairpin RNA, but not of Akt1, blocks the plasmin-induced phosphorylation of ERK1/2 and the chemotactic response. Plasmin-stimulated DCs induce polarization of $CD4^+$ T cells toward the IFN-γ-producing proinflammatory Th1 phenotype [55]. The colocalization of plasmin and DCs in atherosclerotic lesions suggests that plasmin generation in the atherosclerotic vessel wall might contribute to infiltration of DCs, activation of the adaptive immune response, and aggravation of atherosclerosis [55].

At variance to monocytes, macrophages, and DCs, plasmin does not directly activate neutrophils [44, 48]. However, neutrophils can be indirectly activated by plasmin, for example, through the plasmin-mediated activation of perivascular mast cells and the secondary generation of lipid mediators. The plasmin inhibitors, aprotinin, tranexamic, and ϵ-aminocaproic acid, interfere with the activation of mast cells/neutrophils and effectively prevent postischemic neutrophil accumulation, as well as remodeling of the vascular vessel wall [63]. Release of platelet-activating factor by neutrophils after addition of plasmin was also a result of the plasmin-mediated activation of complement cascade [64], thus again, supporting the notion that plasmin does not activate neutrophils directly.

Endothelial cells

The endothelium has many important functions, including maintenance of homeostasis, inhibition of platelet aggregation, and regulation of the vascular tone. It also serves as a platform for leukocyte binding and the process of endothelial transmigration. Yet, pathophysiological stimuli may convert the endothelium into a proinflammatory state, promoting interaction with blood leukocytes and resulting in imbalanced homeostasis. Alterations in endothelial homeostasis promote atherosclerosis and its complications [65]. Similar to monocytes, endothelial cells express the annexin A2 heterotetramer, and they are capable of binding and generating plasmin on their surface [11]. Plasmin, in turn, induces a number of remarkable effects in endothelial cells.

Similar to the situation in monocytes, in endothelial cells, plasmin also specifically induces cleavage of annexin A2 and activation of conventional PKC, and PKC actually phosphorylates two serine residues of annexin A2, leading to dissociation of the heterotetramer complex. These events lead to reduced plasminogen binding and plasmin generation at the endothelial surface. In agreement with these findings, inhibition of conventional PKC leads to a reduction of thrombotic events in a carotid artery injury model, as this treatment maintains the endothelial plasmin generation. Interestingly, plasmin-induced phosphorylation of annexin A2 requires cleavage of A2 and activation of TLR4 on the cell surface. Also the plasmin-induced activation of ERK1/2 in endothelial cells is TLR4-dependent, whereas an involvement of PAR1 has been excluded. These data indicate that plasmin favors, directly or via annexin A2, the activation of TLR4 in the absence of its coreceptor CD14, which is not expressed on endothelial cells [66].

Further direct effects of plasmin on endothelial cells include activation of PLC, stimulation of the arachidonic acid cascade, including prostacyclin biosynthesis [67], as well as induction of integrin $\alpha_V\beta_3$-dependent stress fiber formation [51], cytosolic Ca^{2+} increase, and NO production, which were independent of any PAR1 activation [68]. Moreover, plasmin was reported to inhibit the thrombin-induced signaling via PAR1, implying the proteolytic inactivation of the thrombin receptor [68]. At high concentrations, plasmin induces detachment of the endothelial cell monolayer and when the stimulation persists, initiation of apoptosis [9, 69]. This effect could be mediated by a rapid release of ROS by endothelial cells, as demonstrated in **Fig. 2A**. In addition, similar to LPS, plasmin stimulated production of chemotactic MCP-1 by endothelial cells (Fig. 2B).

Smooth muscle cells

Smooth muscle cells play a key role in the initiation and progression of vascular inflammation. In early stages of atherosclerosis, smooth muscle cells migrate from the media to the intima, where they proliferate. At later stages, they acquire a higher synthetic capacity, leading to exaggerated production of ECM, particularly collagen, proteases, and cytokines, which all contribute to the development of plaques. The stimuli initiating smooth muscle cell migration and proliferation have not been well-elucidated [70]. Plasmin induces proliferation of smooth muscle cells, which is mediated by the GPCR $G_{\alpha i}$. Plasmin induces activation of PI3K/Akt and ERK1/2 and p38 MAPKs in smooth muscle cells. The activation pattern induced by plasmin was distinct from that induced by the uPA [71]. The plasmin-induced proliferation of smooth muscle cells though might depend on the MMP-dependent release of the tethered ligand heparin-binding EGF, which can, in turn, activate the EGFR [72]. Similar to endothelial cells, higher concentrations of plasmin induce detachment and a proteolysis-mediated apoptosis of vascular smooth muscle cells [73].

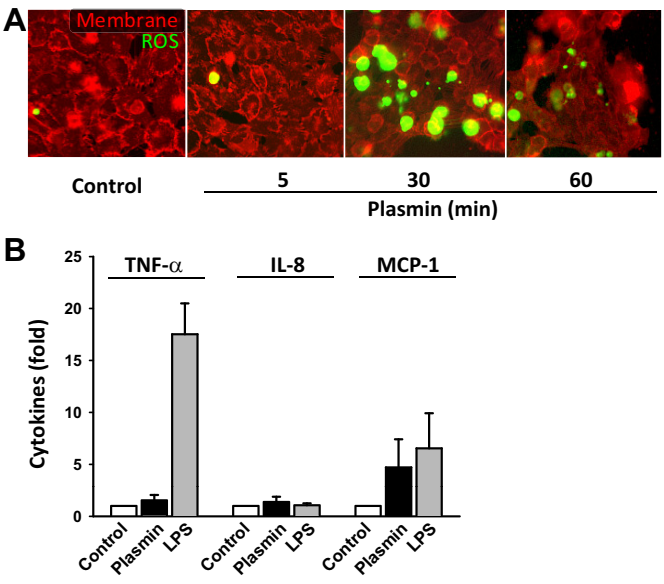

Figure 2. Plasmin-induced activation of endothelial cells. (A) Time-dependent ROS induction by plasmin. HUVECs were labeled with the ROS-sensitive fluorescent dye carboxy-H_2-dichlorofluorescein diacetate, treated with plasmin (0.3 CTA U/ml) for the indicated time, and analyzed by fluorescence microscopy. (B) Human dermal microvascular endothelial cells were stimulated with plasmin (0.143 CTA U/ml) or the positive control LPS (100 ng/ml) for 24 h. The supernatants were recovered, and the concentrations of the cytokines were measured by ELISA (n=3).

Platelets

Activated platelets amplify the inflammatory response through the release of chemokines, cytokines, and other mediators of inflammation, as well as exposure of immunomodulatory ligands on their surface. Platelets aid leukocyte recruiting to sites of vascular injury and inflammation, and they help to maintain and modulate inflammation in atherosclerosis, Alzheimer's disease, and cancer [74]. Plasmin induces various effects on platelets, some of them exhibiting a biphasic mechanism. Thus, plasmin might promote platelet aggregation or might inhibit it depending on concentration [26]. It has been proposed for a long time that the plasmin-induced activation of platelets involves thrombin receptors, supposedly PAR4 [33]. Recently, it has been demonstrated that direct effects of plasmin on platelets, e.g., the intracellular Ca^{2+} increase and platelet aggregation, are mediated by the low-affinity thrombin receptor PAR4. Murine platelets appear to be more sensitive to activation by plasmin compared with human platelets as a result of differences in the extracellular PAR4 sequence, the cleavage of which initiates intracellular signaling events. Different to thrombin-dependent activation of platelets, where PAR3 act as a coreceptor of PAR4, murine PAR3 inhibits a plasmin-induced Ca^{2+} increase and platelet aggregation mediated by PAR4 cleavage and activation [34].

Brain cells

The majority of acute and chronic neurodegenerative diseases is accompanied by local microglia-mediated inflammation [75]. Similar to macrophages in other tissues, microglia, the resident macrophages of the brain, can promote generation of plasmin from plasminogen. The microglia-derived plasmin might exhibit various effects on astrocytes. Thus, plasmin induces activation of prosurvival the PI3K/Akt kinase pathway, leading to production of TGF-β3 by astrocytes [76], which is thought to be important for neuronal survival. As mentioned earlier, plasmin also cleaves proBDNF, leading to generation of the active neurotrophin BDNF, which stimulates neurite outgrowth [77]. Plasmin also induces proteolysis of neuronal NOS, reducing endogenous neuronal NO release, which displays a complex role in neurodegenerative processes, such as neurotoxicity [78]. These data seem to indicate that plasmin might mediate some neuroprotective effects in the CNS. On the other hand, in brain astrocytes, as well as endothelial cells, plasmin induces morphological changes with increased F-actin staining intensity and Rho kinase-dependent elevation of phosphorylated myosin, thereby leading to an increased permeability of the blood-brain barrier [79].

Plasmin triggers hydrolysis of phosphoinositides, an increase in intracellular Ca^{2+}, and an activation of ERK1/2 in cultures of murine cortical astrocytes but not in primary-cultured hippocampal neurons or in CA1 pyramidal cells in hippocampal slices. Plasmin increases the NMDAR-mediated component of miniature excitatory postsynaptic currents in CA1 pyramidal neurons but has no effect on α-amino-3-hydroxy-5-methyl-4-isoxazole propionate- or γ-aminobutyric acid receptor-mediated effects [80]. The effects induced by plasmin in astrocytes are less pronounced in PAR1-deficient cells and could be inhibited by PAR1 antagonists, indicating that plasmin induces activation of murine astrocytes via PAR1 [76, 80].

Keratinocytes

The skin as a primary barrier of the body to the environment is often afflicted by inflammatory diseases. In mice, intradermal injection of plasmin induced a psoriasiform skin inflammation around the injection sites with several aspects of human psoriasis [81]. These effects might be a result of proinflammatory activation of dermal macrophages. However, direct effects of plasmin on keratinocytes have also been documented. Keratinocytes bind plasminogen and plasminogen activators and can initiate plasmin generation on their surface. Plasmin induces directed migration of freshly isolated human epidermal keratinocytes and HaCaT cells in an agarose gel, which was blocked completely by the plasmin inhibitor α_2-antiplasmin, whereas no chemokinesis was detected. Plasmin also enhances the phagocytic killing of *Candida albicans* by freshly isolated epidermal keratinocytes and reduces proliferation of HaCaT keratinocytes, processes that were similarly antagonized by α_2-antiplasmin. These qualities of plasmin might facilitate re-epithelialization following skin injury [82].

Epithelial cells

Plasmin seems to exert direct effects on epithelial cells. Thus, plasmin activates lung epithelial cells to express COX-2 and to produce antifibrotic PGE_2, which modulates collagen deposition by fibrotic fibroblasts [83].

The proper function of epithelial sodium channels is critical for the maintenance of the circulating plasma volume and blood pressure. Proteolytic processing of epithelial sodium channel subunits by plasmin positively modulates activation of the channel current [84]. In patients suffering from nephrotic syndrome, as well as in animal models of proteinuria, the protein-rich urine contains active plasmin, which can activate epithelial sodium channels. These data suggest that a malfunctioning glomerular filtration barrier allows passage of plasminogen, which is transformed to plasmin by uPA. At high concentrations, plasmin directly activates the epithelial sodium channels, whereas at low plasmin concentrations, the channels become activated through an indirect mechanism involving prostasin [85].

In cultured murine tubular epithelial cells, within minutes, plasmin initiated a rapid phosphorylation of ERK1/2 MAPK, followed by phenotypic transition to fibroblast-specific protein-1$^+$, α-smooth muscle actin$^+$, and fibronectin-producing cells. Both processes are apparently mediated by the thrombin receptor PAR1 and are blocked by PAR1 small interfering RNA or by a PAR1 inhibitory peptide. Overexpression of PAR1 enhances the plasmin-mediated, epithelial-mesenchymal transition and ERK1/2 activation [86]. Plasminogen-deficient mice subjected to unilateral ureteral obstruction exhibit significantly lower levels of phosphorylated ERK1/2 and active TGF-β and have reduced total kidney collagen compared with that of WT mice. Epithelial-mesenchymal transition, as typified by tubular loss of E-cadherin and acquisition of α-smooth muscle actin, is also impaired significantly in plasminogen-deficient mice, indicating that plasmin might influence the renal function [86].

Fibroblasts

Mechanical, chemical, and microbial irritation of the dental pulp can provoke inflammatory processes. It has been claimed that plasmin plays an important role in pulpal disease, which is associated with tissue degradation. In human dental pulp fibroblast-like cells, plasmin increases the cytosolic calcium concentration and the expression of the proinflammatory mediators PGE_2 and IL-8 [87]. The concentrations of plasmin used in this study were high enough (100 μM) to induce cleavage and activation of the thrombin receptor PAR1. Accordingly, the plasmin-induced effects were inhibited by a PAR1 inhibitor. However, the relevance of these findings for pulpitis remains to be established. As to pulmonary diseases, plasmin might stimulate fibroblasts, as well as alveolar epithelial cells, to express COX-2 and to release antifibrotic PGE_2 protecting from lung fibrosis [83]. This process is indeed mediated by plasmin and is amplified in cells from plasminogen activator inhibitor-deficient mice. In this setting, the plasmin-induced activation of human fibroblasts is independent of any PAR1 signaling. Fibroblasts from patients with idiopathic pulmonary fibrosis are defective in their ability to induce COX-2 and to up-regulate PGE_2 synthesis in response to plasmin [83]. The median survival of these patients is only approximately 3 years. They exhibit low lung levels of PGE_2, and their fibroblasts are resistant to the PGE_2-mediated inhibition of collagen synthesis by fibroblasts. Further studies by the same authors demonstrated that plasmin induces PKA activation and inhibition of protein phosphatase 2A, which restores the sensitivity of fibrotic lung fibroblasts from humans and mice to PGE_2 [88], suggesting plasmin as a new therapeutic strategy to control collagen deposition by fibrotic fibroblasts (**Table 1**).

PLASMINOGEN-PLASMIN BALANCE IN DISEASES

Consistent with the central role of plasmin in dissolution of fibrin clots, plasma hypofibrinolysis is a risk factor for venous thrombosis, whereas enhanced fibrinolysis is associated with bleeding disorders [11]. No significantly increased risk of deep venous thrombosis was, however, observed in hypoplasminogenemia, indicating that up to certain levels, the fibrinolytic imbalance could be well-compensated [89]. In addition to dissolution of fibrin clots, plasmin has many other substrates and functions, which might be impaired when the blood fibrinolytic activity is severely affected. Thus, the fibrinolytic equilibrium is shifted during various pathological conditions. Plasmin activity is, for example, decreased in infection, and a low plasmin activity correlates with poor prognosis in severe sepsis [90, 91]. Additional inhibition of plasmin activity by a plasmin inhibitor aggravates severity and mortality in a murine model of staphylococcal arthritis and sepsis [90]. These findings are somewhat unexpected, as the dissemination of bacteria, as has been shown in *Borrelia burgdorferi* infection in plasminogen-deficient mice and in group A *Streptococci*-infected mice, critically depends on plasmin activity [11, 89]. Accordingly, plasminogen-deficient mice exhibit increased resistance to *Yersinia pestis* (plague) compared with WT mice [89].

Plasmin-induced activation of immune cells, such as macrophages and monocytes, might be important for the successful elimination of the bacterial burden. This hypothesis was supported by studies of *Staphylococcus aureus*-induced bacterial arthritis and infection in plasminogen-deficient mice [92, 93]. Thus, activation of neutrophils and macrophages as well as expression of IL-6 and IL-10 in plasminogen-deficient mice are markedly reduced. This is associated with an increased bacterial burden in joints of plasminogen-deficient mice, whereas in WT littermates, the bacteria are eliminated by Day 28. Supplementation with plasminogen reverses the reduced release of IL-6. Interestingly, recruitment of macrophages and neutrophils to infected joints is not affected by plasminogen deficiency; only the activation of immune cells and the cytokine release are impaired [92]. Similarly, plasminogen-deficient mice exhibit higher mortality in *S. aureus* infection, possibly as a result of the reduced release of proinflammatory cytokines, which aid the elimination of bacteria [93]. However, when high amounts of bacteria are injected i.v. in this murine sepsis model, the reduced cytokine levels in plasminogen-deficient mice are protective, cause less tissue destruction, and reduce markedly the mortality of these mice [93]. Likewise, plasmin may have protective effects in specific murine arthritis models associated with local joint trauma, where plasmin appears to promote wound healing and remodeling [94]. In collagen type II-induced arthritis though, plasmin is indispensable for the disease progression [95]. The equivocal role of plasmin

TABLE 1. Plasmin-Induced Cell Activation

Cell type	Effect on cellular function	Receptor	Plasmin concentration	Reference
Monocytes	Chemotaxis	Annexin A2 heterotetramer PAR1-independent	0.01–1.1 μM; 0.01–1.4 CTA U/ml	[43, 50]
Monocytes	TNF-α, IL-1α, IL-1β, CD40, tissue factor, MCP-1	—	0.03–1.1 μM; 0.04–1.4 CTA U/ml	[41, 49]
Monocytes	Cysteinyl-leukotriene	—	0.001–1.0 casein units/ml	[44, 48]
Monocytes	Antiapoptotic	PAR1	0.5–2 μM plasminogen	[54]
Monocytes	COX-2, PGE_2, MMP-1	Annexin A2 heterotetramer	0.1–0.36 μM	[52]
Macrophages	Chemotaxis, TNF-α, IL-6	Annexin A2 heterotetramer	0.33 μM; 0.4 CTA U/ml	[58]
U937 monocytic cells	Chemotaxis, invasion	Plg-R_{KT} (partially)	0.2–2.0 μM plasminogen	[30]
RAW264.7 macrophages	TNF-α, IL-8	PAR-independent	0.01–1.0 U/ml	[61]
DCs	Chemotaxis	Annexin A2 heterotetramer	0.01–1.1 μM; 0.01–1.4 CTA U/ml	[55]
Endothelial cells	PKC-dependent cell-surface plasmin generation	Annexin A2 heterotetramer; TLR4 PAR1-independent	0.04–0.86 μM	[66]
Endothelial cells	$[Ca^{2+}]_i$, prostacyclin, arachidonic acid	—	0.01–1.0 μM	[67]
Endothelial cells	Stress fiber formation	$\alpha_V\beta_3$	0.05–0.2 μM	[51]
Endothelial cells	$[Ca^{2+}]_i$, NO	PAR1-independent	0.01–1.0 μM	[68]
Endothelial cells	Apoptosis	—	0.1–2.0 μM plasminogen	[69]
Smooth muscle cells	Proliferation	$G\alpha_i$ uPAR-independent	100.0 U/ml	[71]
Smooth muscle cells	Apoptosis	—	1.25 μM plasminogen	[73]
Platelets	Aggregation, low $[Ca^{2+}]_i$	PAR4 PAR1-independent	1.0 U/ml	[33]
Platelets	Aggregation, $[Ca^{2+}]_i$	PAR4 PAR3-independent	0.01–1.0 U/ml	[34]
Astrocytes	$[Ca^{2+}]_i$, TGF-β3	PAR1	0.01–0.1 μM plasminogen	[76]
Astrocytes	Actin polymerization	—	0.02 μM plasminogen	[79]
Astrocytes	$[Ca^{2+}]_i$, changes in excitatory postsynaptic currents	PAR1	0.1–1.0 μM	[80]
Keratinocytes	Chemotaxis, phagocytic killing *C. albicans*	—	0.01–0.05 U/ml	[82]
Epithelial cells	COX-2, PGE_2	—	0.01–0.1 U/ml	[83]
Epithelial cells	Activation of Na^+ channels	—	0.03–0.3 μM	[84, 85]
Epithelial cells	Epithelial-mesenchymal transition	PAR1	0.22 μM	[86]
Fibroblasts	$[Ca^{2+}]_i$, PGE_2, IL-8	PAR1	100.0 μM	[87]
Fibroblasts	COX-2, PGE_2	PAR1-independent	0.01–0.1 U/ml	[83]

in different mouse models of arthritis has been summarized comprehensively elsewhere [96].

It should be noted that in LPS-induced inflammation in vivo in human subjects, plasmin inhibition by the lysine analog tranexamic acid had almost no effect on the plasma levels of cytokines (TNF-α, IL-6, IL-10, and IL-8), although plasmin generation and fibrin degradation were inhibited successfully [97]. The obvious discrepancy to the experiments with the plasminogen-deficient mice might be explained by the nature of plasmin generation and inhibition by the lysine analog. It is plausible that tranexamic acid in the concentration used effectively inhibits free plasmin and plasmin-mediated fibrin cleavage but may have less effect on the cell-bound, especially the monocyte membrane-bound, plasmin. Monocytes are the major source of blood cytokines, and they express the machinery required for plasmin activation on their cell surface [26]. Moreover, stimulation with LPS or cytokines leads to increased expression of the uPAR on the monocyte surface [98]. Approximately 10 μg/ml tranexamic acid is sufficient to inhibit fibrinolysis [99]. In our studies, up to 80 μg/ml tranexamic acid was not sufficient to completely inhibit the plasmin-induced cysteinyl-leukotriene synthesis [44] and chemotaxis [43] of human monocytes. In addition, plasma concentrations of tranexamic acid decrease rapidly after infusion ($t_{½}$~1 h) [100], whereas the plasmin generation on the monocyte surface may be delayed.

Plasmin is important for the infiltration of immune cells and the progression of inflammation. Thus, no inflammation was observed in plasminogen-deficient mice injected with collagen type II and anticollagen type II mAb to induce autoimmune arthritis, unless the mice were substituted i.v. with plasminogen [95]. These examples illustrate the role of plasmin in lo-

cal and systemic inflammatory responses. Hence, inhibition of plasmin and kallikrein with aprotinin after cardiopulmory bypass attenuates the overall systemic inflammatory reaction in the patients associated with cardiac surgery [101].

In acute and chronic inflammatory lung diseases, the hemostatic balance is compromised, and the fibrinolytic activity is decreased as a result of inhibition of plasminogen activators by the plasminogen activator inhibitor, as well as blockage of plasmin by the α_2-plasmin inhibitor, leading to excessive deposition of intra-alveolar fibrin [102]. However, in experimental asthma, lung inflammation, IL-5, and TNF-α in BAL fluid, perivascular leukocytosis, mucus production, and collagen deposition are all reduced in plasminogen-deficient mice [103].

Plasmin appears to have equivocal roles in cardiovascular diseases. It is well-established that the expression of fibrinolytic genes is increased in the atherosclerotic aorta, and the up-regulated fibrinolytic activity correlates with the severity of coronary lesions [26]. A recent study revealed enhanced plasmin activity in human carotid plaques, which correlates with the degree of lesion complexity [104]. Although the presence of proteases and plasmin in atherosclerosis has been regarded mainly in terms of plaque destabilization, plasmin-activated macrophages play an important role in the pathogenesis of cardiovascular disease. A clinical case report presented a patient with heterozygous α_2-antiplasmin deficiency, who developed atherosclerosis and myocardial ischemia [105], suggesting that aggravated plasmin generation might indeed lead to cardiovascular complications. However, the effects of plasmin might be versatile [11, 26]. Also, deficiency in the plasminogen activator inhibitor may enhance lesion formation, as cells that invade the developing plaque may require plasmin activity for their directed migration. For example, in mouse models, atherosclerotic plague formation is increased in plasminogen-deficient animals. On the other hand, the development of atherosclerosis is reduced markedly in a transplant model in the plasminogen-deficient mice [11, 26]. Similarly, α_2-antiplasmin deficiency, which leads to enhanced plasmin generation in vivo, has no effect on neointima formation in mice [106]. These effects might, however, be mediated by various plasmin substrates, such as TGF-β1, MMPs, and others [26, 106]. The lack of consistency in studies performed on vascular remodeling and atherosclerosis in animal models could also be a result of opposing effects of plasmin on different cell types involved in tissue repair and remodeling, as well as genetically modified mice models with entire deficiency of various fibrinolytic factors. The former consideration is supported by a study, where the macrophage-targeted overexpression of uPA accelerated atherosclerosis, although as mentioned above, studies in genetically modified mice may favor an antiatherogenic role of plasmin/ogen and its activator [106, 107].

There might be another substantial difference between plasmin-induced effects in animal models and humans. Most effects, mediated by plasmin in human cells, occur independent of PAR activation, and plasmin is known to cleave and desensitize human PAR1 [108]. However, higher plasmin concentrations might also lead to PAR1 activation on human monocytes [54] and PAR4 on human platelets [33, 34]. In mice, the situation might be different. Low plasmin concentrations are sufficient to activate PAR4 on murine platelets, which at variance to human platelets, do not express PAR1 [34], as well as PAR1 on murine astrocytes [80]. Although in many studies, human plasmin was used to activate murine cells, which may not truly reflect the in vivo situation, in the latter study, murine plasmin and PAR1-deficient mice were used to confirm the conclusions [80]. In the context of renal fibrosis, plasmin-induced epithelial-to-mesenchymal transition in mice is also dependent on PAR1signaling [86]. Similarly, in rat astrocytes, rat plasmin induces TGF-β3 release, which is inhibited by a PAR1 inhibitor [76]. These data suggest that in rodent models, plasmin-induced effects might be mediated by receptor(s) different from those in humans. Indeed, significant differences between murine and human plasminogen activation systems have been discussed previously [11, 106]. Therefore, any extrapolation of

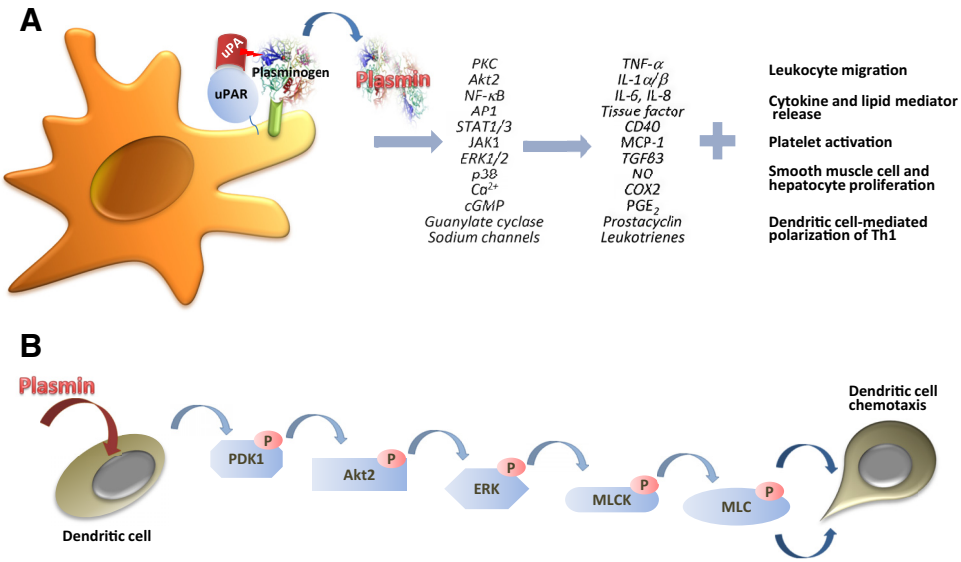

Figure 3. Plasmin-induced proinflammatory cell activation. (A) Signaling pathways and mediators activated by plasmin. (B) Plasmin-induced kinase cascade leading to chemotaxis of human DCs. PDK1, Phosphoinositide-dependent kinase-1; MLCK, myosin light-chain kinase; P, phosphorylated.

data from transgenic murine models to humans requires very careful consideration.

CONCLUDING REMARKS

Data from in vitro and in vivo studies imply the ability of plasmin to induce activation of inflammatory cells and to stimulate the production of cytokines, free radical species, and other mediators. The plasmin-induced activation of monocytes, macrophages, and DCs reveals that it is a potent stimulus and chemoattractant of cells playing a central role in immune cell response, which aids elimination of infectious organisms, tissue regeneration, and healing (**Fig. 3**). However, excessive activation of plasmin in chronic inflammation or autoimmune diseases might contribute to maintain or exacerbate the pathogenesis of the respective disease.

AUTHORSHIP

T. Syrovets and T. Simmet wrote the paper. O.L. performed ROS measurements and other experiments.

ACKNOWLEDGMENTS

The work was supported by the German Research Foundation (DFG).

REFERENCES

1. Booth, N. A., Bachman, F. (2006) Plasminogen-plasmin system. In *Hemostasis and Thrombosis: Basic Principles and Clinical Practice* (W. Colman, R. J. Marder, V. W. Clowes, A. N. George, J. Z. Goldhaber, eds.), S. Lippincott Williams & Wilkins, Philadelphia, PA, USA, 335–365.
2. Petersen, T. E., Martzen, M. R., Ichinose, A., Davie, E. W. (1990) Characterization of the gene for human plasminogen, a key proenzyme in the fibrinolytic system. *J. Biol. Chem.* **265,** 6104–6111.
3. Wu, T. P., Padmanabhan, K. P., Tulinsky, A. (1994) The structure of recombinant plasminogen kringle 1 and the fibrin binding site. *Blood Coagul. Fibrinolysis* **5,** 157–166.
4. Rios-Steiner, J. L., Schenone, M., Mochalkin, I., Tulinsky, A., Castellino, F. J. (2001) Structure and binding determinants of the recombinant kringle-2 domain of human plasminogen to an internal peptide from a group A Streptococcal surface protein. *J. Mol. Biol.* **308,** 705–719.
5. Christen, M. T., Frank, P., Schaller, J., Llinas, M. (2010) Human plasminogen kringle 3: solution structure, functional insights, phylogenetic landscape. *Biochemistry* **49,** 7131–7150.
6. Mulichak, A. M., Tulinsky, A., Ravichandran, K. G. (1991) Crystal and molecular structure of human plasminogen kringle 4 refined at 1.9-A resolution. *Biochemistry* **30,** 10576–10588.
7. Battistel, M. D., Grishaev, A., An, S. S., Castellino, F. J., Llinas, M. (2009) Solution structure and functional characterization of human plasminogen kringle 5. *Biochemistry* **48,** 10208–10219.
8. Peisach, E., Wang, J., de los Santos, T., Reich, E., Ringe, D. (1999) Crystal structure of the proenzyme domain of plasminogen. *Biochemistry* **38,** 11180–11188.
9. Ho-Tin-Noe, B., Rojas, G., Vranckx, R., Lijnen, H. R., Angles-Cano, E. (2005) Functional hierarchy of plasminogen kringles 1 and 4 in fibrinolysis and plasmin-induced cell detachment and apoptosis. *FEBS J.* **272,** 3387–3400.
10. Miles, L. A., Dahlberg, C. M., Plow, E. F. (1988) The cell-binding domains of plasminogen and their function in plasma. *J. Biol. Chem.* **263,** 11928–11934.
11. Cesarman-Maus, G., Hajjar, K. A. (2005) Molecular mechanisms of fibrinolysis. *Br. J. Haematol.* **129,** 307–321.
12. Miles, L. A., Hawley, S. B., Baik, N., Andronicos, N. M., Castellino, F. J., Parmer, R. J. (2005) Plasminogen receptors: the sine qua non of cell surface plasminogen activation. *Front. Biosci.* **10,** 1754–1762.
13. Dejouvencel, T., Doeuvre, L., Lacroix, R., Plawinski, L., Dignat-George, F., Lijnen, H. R., Angles-Cano, E. (2010) Fibrinolytic cross-talk: a new mechanism for plasmin formation. *Blood* **115,** 2048–2056.
14. Colman, R. W. (2006) Contact activation (kallikrein-kinin) pathway: multiple physiologic and pathophysiologic activities. In *Hemostasis and Thrombosis: Basic Principles and Clinical Practice* (W. Colman, R. J. Marder, V. W. Clowes, A. N. George, J. Z. Goldhaber, eds.), S. Lippincott Williams & Wilkins, Philadelphia, PA, USA, 107–130.
15. Gailani, D., Renne, T. (2007) The intrinsic pathway of coagulation: a target for treating thromboembolic disease? *J. Thromb. Haemost.* **5,** 1106–1112.
16. Banbula, A., Zimmerman, T. P., Novokhatny, V. V. (2007) Blood inhibitory capacity toward exogenous plasmin. *Blood Coagul. Fibrinolysis* **18,** 241–246.
17. Marder, V. J., Francis, C. W. (2006) Physiologic regualtion of fibrinolysis. In *Hemostasis and Thrombosis: Basic Principles and Clinical Practice* (W. Colman, R. J. Marder, V. W. Clowes, A. N. George, J. Z. Goldhaber, eds.), S. Lippincott Williams & Wilkins, Philadelphia, PA, USA, 419–436.
18. Lacroix, R., Sabatier, F., Mialhe, A., Basire, A., Pannell, R., Borghi, H., Robert, S., Lamy, E., Plawinski, L., Camoin-Jau, L., Gurewich, V., Angles-Cano, E., Dignat-George, F. (2007) Activation of plasminogen into plasmin at the surface of endothelial microparticles: a mechanism that modulates angiogenic properties of endothelial progenitor cells in vitro. *Blood* **110,** 2432–2439.
19. Weinstein, M. J., Doolittle, R. F. (1972) Differential specificities of the thrombin, plasmin and trypsin with regard to synthetic and natural substrates and inhibitors. *Biochim. Biophys. Acta* **258,** 577–590.
20. Amara, U., Flierl, M. A., Rittirsch, D., Klos, A., Chen, H., Acker, B., Bruckner, U. B., Nilsson, B., Gebhard, F., Lambris, J. D., Huber-Lang, M. (2010) Molecular intercommunication between the complement and coagulation systems. *J. Immunol.* **185,** 5628–5636.
21. Hashimoto, K. (2010) Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. *Psychiatry Clin. Neurosci.* **64,** 341–357.
22. Roth, D., Piekarek, M., Paulsson, M., Christ, H., Bloch, W., Krieg, T., Davidson, J. M., Eming, S. A. (2006) Plasmin modulates vascular endothelial growth factor-A-mediated angiogenesis during wound repair. *Am. J. Pathol.* **168,** 670–684.
23. Heissig, B., Ohki, M., Ishihara, M., Tashiro, Y., Nishida, C., Gritli, I., Rosenkvist, J., Hattori, K. (2009) Contribution of the fibrinolytic pathway to hematopoietic regeneration. *J. Cell. Physiol.* **221,** 521–525.
24. Grabowska, M. M., Day, M. L. (2012) Soluble E-cadherin: more than a symptom of disease. *Front. Biosci.* **17,** 1948–1964.
25. Mortier, A., Berghmans, N., Ronsse, I., Grauwen, K., Stegen, S., Van Damme, J., Proost, P. (2011) Biological activity of CXCL8 forms generated by alternative cleavage of the signal peptide or by aminopeptidase-mediated truncation. *PLoS One* **6,** e23913.
26. Syrovets, T., Simmet, T. (2004) Novel aspects and new roles for the serine protease plasmin. *Cell. Mol. Life Sci.* **61,** 873–885.
27. Madureira, P. A., Surette, A. P., Phipps, K. D., Taboski, M. A., Miller, V. A., Waisman, D. M. (2011) The role of the annexin A2 heterotetramer in vascular fibrinolysis. *Blood* **118,** 4789–4797.
28. Herren, T., Swaisgood, C., Plow, E. F. (2003) Regulation of plasminogen receptors. *Front. Biosci.* **8,** D1–D8.
29. Andronicos, N. M., Chen, E. I., Baik, N., Bai, H., Parmer, C. M., Kiosses, W. B., Kamps, M. P., Yates III, J. R., Parmer, R. J., Miles, L. A. (2010) Proteomics-based discovery of a novel, structurally unique, and developmentally regulated plasminogen receptor, Plg-RKT, a major regulator of cell surface plasminogen activation. *Blood* **115,** 1319–1330.
30. Lighvani, S., Baik, N., Diggs, J. E., Khaldoyanidi, S., Parmer, R. J., Miles, L. A. (2011) Regulation of macrophage migration by a novel plasminogen receptor Plg-R KT. *Blood* **118,** 5622–5630.
31. Adams, M. N., Ramachandran, R., Yau, M. K., Suen, J. Y., Fairlie, D. P., Hollenberg, M. D., Hooper, J. D. (2011) Structure, function and pathophysiology of protease activated receptors. *Pharmacol. Ther.* **130,** 248–282.
32. Coughlin, S. R. (2005) Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J. Thromb. Haemost.* **3,** 1800–1814.
33. Quinton, T. M., Kim, S., Derian, C. K., Jin, J., Kunapuli, S. P. (2004) Plasmin-mediated activation of platelets occurs by cleavage of protease-activated receptor 4. *J. Biol. Chem.* **279,** 18434–18439.
34. Mao, Y., Jin, J., Daniel, J. L., Kunapuli, S. P. (2009) Regulation of plasmin-induced protease-activated receptor 4 activation in platelets. *Platelets* **20,** 191–198.
35. Gaestel, M., Kotlyarov, A., Kracht, M. (2009) Targeting innate immunity protein kinase signalling in inflammation. *Nat. Rev. Drug Discov.* **8,** 480–499.
36. Gordon, S. (1999) Development and distribution of mononuclear phagocytes: relevance to inflammation. In *Inflammation: Basic Principles and Clinical Correlates* (I. Gallin, J. Snyderman, R., eds.), Lippincott Williams & Wilkins, Philadelphia, PA, USA, 35–48.
37. Shi, C., Pamer, E. G. (2011) Monocyte recruitment during infection and inflammation. *Nat. Rev. Immunol.* **11,** 762–774.
38. Weber, C., Noels, H. (2011) Atherosclerosis: current pathogenesis and therapeutic options. *Nat. Med.* **17,** 1410–1422.
39. Simmet, T., Luck, W. (1989) Clotting of whole human blood induces cysteinyl-leukotriene formation. *Thromb. Res.* **54,** 423–433.

40. Simmet, T., Weide, I. (1991) Thromboxane and cysteinyl-leukotriene formation are differentially activated in spontaneously clotting whole human blood in vitro. *Thromb. Res.* **62,** 249–261.
41. Syrovets, T., Jendrach, M., Rohwedder, A., Schule, A., Simmet, T. (2001) Plasmin-induced expression of cytokines and tissue factor in human monocytes involves AP-1 and IKKβ-mediated NF-κB activation. *Blood* **97,** 3941–3950.
42. Syrovets, T., Schule, A., Jendrach, M., Büchele, B., Simmet, T. (2002) Ciglitazone inhibits plasmin-induced proinflammatory monocyte activation via modulation of p38 MAP kinase activity. *Thromb. Haemost.* **88,** 274–281.
43. Syrovets, T., Tippler, B., Rieks, M., Simmet, T. (1997) Plasmin is a potent and specific chemoattractant for human peripheral monocytes acting via a cyclic guanosine monophosphate-dependent pathway. *Blood* **89,** 4574–4583.
44. Weide, I., Römisch, J., Simmet, T. (1994) Contact activation triggers stimulation of the monocyte 5-lipoxygenase pathway via plasmin. *Blood* **83,** 1941–1951.
45. Weide, I., Simmet, T. (1993) Leukotriene formation by peripheral monocytes in contact-activated human blood. *Thromb. Res.* **71,** 185–192.
46. Weide, I., Simmet, T. (1995) Novel mode of monocyte 5-lipoxygenase stimulation. *Adv. Prostaglandin Thromboxane Leukot. Res.* **23,** 325–327.
47. Weide, I., Simmet, T. (1995) Contact-mediated stimulation of the monocyte 5-lipoxygenase pathway is enhanced by the presence of platelets. *Thromb. Res.* **77,** 187–192.
48. Weide, I., Tippler, B., Syrovets, T., Simmet, T. (1996) Plasmin is a specific stimulus of the 5-lipoxygenase pathway of human peripheral monocytes. *Thromb. Haemost.* **76,** 561–568.
49. Burysek, L., Syrovets, T., Simmet, T. (2002) The serine protease plasmin triggers expression of MCP-1 and CD40 in human primary monocytes via activation of p38 MAPK and janus kinase (JAK)/STAT signaling pathways. *J. Biol. Chem.* **277,** 33509–33517.
50. Laumonnier, Y., Syrovets, T., Burysek, L., Simmet, T. (2006) Identification of the annexin A2 heterotetramer as a receptor for the plasmin-induced signaling in human peripheral monocytes. *Blood* **107,** 3342–3349.
51. Tarui, T., Majumdar, M., Miles, L. A., Ruf, W., Takada, Y. (2002) Plasmin-induced migration of endothelial cells. A potential target for the anti-angiogenic action of angiostatin. *J. Biol. Chem.* **277,** 33564–33570.
52. Zhang, Y., Zhou, Z. H., Bugge, T. H., Wahl, L. M. (2007) Urokinase-type plasminogen activator stimulation of monocyte matrix metalloproteinase-1 production is mediated by plasmin-dependent signaling through annexin A2 and inhibited by inactive plasmin. *J. Immunol.* **179,** 3297–3304.
53. Yona, S., Jung, S. (2010) Monocytes: subsets, origins, fates and functions. *Curr. Opin. Hematol.* **17,** 53–59.
54. Mitchell, J. W., Baik, N., Castellino, F. J., Miles, L. A. (2006) Plasminogen inhibits TNFα-induced apoptosis in monocytes. *Blood* **107,** 4383–4390.
55. Li, X., Syrovets, T., Genze, F., Pitterle, K., Oberhuber, A., Orend, K. H., Simmet, T. (2010) Plasmin triggers chemotaxis of monocyte-derived dendritic cells through an Akt2-dependent pathway and promotes a T-helper type-1 response. *Arterioscler. Thromb. Vasc. Biol.* **30,** 582–590.
56. Phipps, K. D., Surette, A. P., O'Connell, P. A., Waisman, D. M. (2011) Plasminogen receptor S100A10 is essential for the migration of tumor-promoting macrophages into tumor sites. *Cancer Res.* **71,** 6676–6683.
57. Kumar, H., Kawai, T., Akira, S. (2009) Toll-like receptors and innate immunity. *Biochem. Biophys. Res. Commun.* **388,** 621–625.
58. Li, Q., Laumonnier, Y., Syrovets, T., Simmet, T. (2007) Plasmin triggers cytokine induction in human monocyte-derived macrophages. *Arterioscler. Thromb. Vasc. Biol.* **27,** 1383–1389.
59. Swisher, J. F., Burton, N., Bacot, S. M., Vogel, S. N., Feldman, G. M. (2010) Annexin A2 tetramer activates human and murine macrophages through TLR4. *Blood* **115,** 549–558.
60. Swisher, J. F., Khatri, U., Feldman, G. M. (2007) Annexin A2 is a soluble mediator of macrophage activation. *J. Leukoc. Biol.* **82,** 1174–1184.
61. Ward, J. R., Dower, S. K., Whyte, M. K., Buttle, D. J., Sabroe, I. (2006) Potentiation of TLR4 signalling by plasmin activity. *Biochem. Biophys. Res. Commun.* **341,** 299–303.
62. Buckwalter, M. R., Albert, M. L. (2009) Orchestration of the immune response by dendritic cells. *Curr. Biol.* **19,** R355–R361.
63. Reichel, C. A., Lerchenberger, M., Uhl, B., Rehberg, M., Berberich, N., Zahler, S., Wymann, M. P., Krombach, F. (2011) Plasmin inhibitors prevent leukocyte accumulation and remodeling events in the postischemic microvasculature. *PLoS One* **6,** e17229.
64. Lupia, E., Del Sorbo, L., Bergerone, S., Emanuelli, G., Camussi, G., Montrucchio, G. (2003) The membrane attack complex of complement contributes to plasmin-induced synthesis of platelet-activating factor by endothelial cells and neutrophils. *Immunology* **109,** 557–563.
65. Pober, J. S., Sessa, W. C. (2007) Evolving functions of endothelial cells in inflammation. *Nat. Rev. Immunol.* **7,** 803–815.
66. He, K. L., Sui, G., Xiong, H., Broekman, M. J., Huang, B., Marcus, A. J., Hajjar, K. A. (2011) Feedback regulation of endothelial cell surface plasmin generation by PKC-dependent phosphorylation of annexin A2. *J. Biol. Chem.* **286,** 15428–15439.
67. Chang, W. C., Shi, G. Y., Chow, Y. H., Chang, L. C., Hau, J. S., Lin, M. T., Jen, C. J., Wing, L. Y., Wu, H. L. (1993) Human plasmin induces a receptor-mediated arachidonate release coupled with G proteins in endothelial cells. *Am. J. Physiol.* **264,** C271–C281.
68. Fujiyoshi, T., Hirano, K., Hirano, M., Nishimura, J., Takahashi, S., Kanaide, H. (2007) Plasmin induces endothelium-dependent nitric oxide-mediated relaxation in the porcine coronary artery. *Arterioscler. Thromb. Vasc. Biol.* **27,** 949–954.
69. Doeuvre, L., Plawinski, L., Goux, D., Vivien, D., Angles-Cano, E. (2010) Plasmin on adherent cells: from microvesiculation to apoptosis. *Biochem. J.* **432,** 365–373.
70. Rautou, P. E., Vion, A. C., Amabile, N., Chironi, G., Simon, A., Tedgui, A., Boulanger, C. M. (2011) Microparticles, vascular function, and atherothrombosis. *Circ. Res.* **109,** 593–606.
71. Nicholl, S. M., Roztocil, E., Galaria, I. I., Davies, M. G. (2005) Plasmin induces smooth muscle cell proliferation. *J. Surg. Res.* **127,** 39–45.
72. Roztocil, E., Nicholl, S. M., Galaria, I. I., Davies, M. G. (2005) Plasmin-induced smooth muscle cell proliferation requires epidermal growth factor activation through an extracellular pathway. *Surgery* **138,** 180–186.
73. Rossignol, P., Angles-Cano, E., Lijnen, H. R. (2006) Plasminogen activator inhibitor-1 impairs plasminogen activation-mediated vascular smooth muscle cell apoptosis. *Thromb. Haemost.* **96,** 665–670.
74. Nurden, A. T. (2011) Platelets, inflammation and tissue regeneration. *Thromb. Haemost.* **105** (Suppl. 1), S13–S33.
75. Kaminska, B., Gozdz, A., Zawadzka, M., Ellert-Miklaszewska, A., Lipko, M. (2009) MAPK signal transduction underlying brain inflammation and gliosis as therapeutic target. *Anat. Rec. (Hoboken)* **292,** 1902–1913.
76. Maeda, S., Nakajima, K., Tohyama, Y., Kohsaka, S. (2009) Characteristic response of astrocytes to plasminogen/plasmin to upregulate transforming growth factor β 3 (TGFβ3) production/secretion through proteinase-activated receptor-1 (PAR-1) and the downstream phosphatidylinositol 3-kinase (PI3K)-Akt/PKB signaling cascade. *Brain Res.* **1305,** 1–13.
77. Gray, K., Ellis, V. (2008) Activation of pro-BDNF by the pericellular serine protease plasmin. *FEBS Lett.* **582,** 907–910.
78. Baron, A., Hommet, Y., Casse, F., Vivien, D. (2010) Tissue-type plasminogen activator induces plasmin-dependent proteolysis of intracellular neuronal nitric oxide synthase. *Biol. Cell* **102,** 539–547.
79. Niego, B., Freeman, R., Puschmann, T. B., Turnley, A. M., Medcalf, R. L. (2012) t-PA-specific modulation of a human BBB model involves plasmin-mediated activation of the Rho-kinase pathway in astrocytes. *Blood*, doi:10.1182/blood-2011-07-369512 [Epub ahead of print].
80. Mannaioni, G., Orr, A. G., Hamill, C. E., Yuan, H., Pedone, K. H., McCoy, K. L., Berlinguer Palmini, R., Junge, C. E., Lee, C. J., Yepes, M., Hepler, J. R., Traynelis, S. F. (2008) Plasmin potentiates synaptic N-methyl-D-aspartate receptor function in hippocampal neurons through activation of protease-activated receptor-1. *J. Biol. Chem.* **283,** 20600–20611.
81. Li, Q., Ke, F., Zhang, W., Shen, X., Xu, Q., Wang, H., Yu, X. Z., Leng, Q. (2011) Plasmin plays an essential role in amplification of psoriasiform skin inflammation in mice. *PLoS One* **6,** e16483.
82. Szabo, I., Simon Jr., M., Hunyadi, J. (2004) Plasmin promotes keratinocyte migration and phagocytic-killing accompanied by suppression of cell proliferation which may facilitate re-epithelialization of wound beds. *Clin. Dev. Immunol.* **11,** 233–240.
83. Bauman, K. A., Wettlaufer, S. H., Okunishi, K., Vannella, K. M., Stoolman, J. S., Huang, S. K., Courey, A. J., White, E. S., Hogaboam, C. M., Simon, R. H., Toews, G. B., Sisson, T. H., Moore, B. B., Peters-Golden, M. (2010) The antifibrotic effects of plasminogen activation occur via prostaglandin E2 synthesis in humans and mice. *J. Clin. Invest.* **120,** 1950–1960.
84. Passero, C. J., Mueller, G. M., Rondon-Berrios, H., Tofovic, S. P., Hughey, R. P., Kleyman, T. R. (2008) Plasmin activates epithelial Na+ channels by cleaving the γ subunit. *J. Biol. Chem.* **283,** 36586–36591.
85. Svenningsen, P., Friis, U. G., Bistrup, C., Buhl, K. B., Jensen, B. L., Skott, O. (2011) Physiological regulation of epithelial sodium channel by proteolysis. *Curr. Opin. Nephrol. Hypertens.* **20,** 529–533.
86. Zhang, G., Kernan, K. A., Collins, S. J., Cai, X., Lopez-Guisa, J. M., Degen, J. L., Shvil, Y., Eddy, A. A. (2007) Plasmin(ogen) promotes renal interstitial fibrosis by promoting epithelial-to-mesenchymal transition: role of plasmin-activated signals. *J. Am. Soc. Nephrol.* **18,** 846–859.
87. Kamio, N., Hashizume, H., Nakao, S., Matsushima, K., Sugiya, H. (2008) Plasmin is involved in inflammation via protease-activated receptor-1 activation in human dental pulp. *Biochem. Pharmacol.* **75,** 1974–1980.
88. Okunishi, K., Sisson, T. H., Huang, S. K., Hogaboam, C. M., Simon, R. H., Peters-Golden, M. (2011) Plasmin overcomes resistance to prostaglandin E2 in fibrotic lung fibroblasts by reorganizing protein kinase A signaling. *J. Biol. Chem.* **286,** 32231–32243.
89. Schuster, V., Hugle, B., Tefs, K. (2007) Plasminogen deficiency. *J. Thromb. Haemost.* **5,** 2315–2322.

90. Klak, M., Anakkala, N., Wang, W., Lange, S., Jonsson, I. M., Tarkowski, A., Jin, T. (2010) Tranexamic acid, an inhibitor of plasminogen activation, aggravates staphylococcal septic arthritis and sepsis. *Scand. J. Infect. Dis.* **42,** 351–358.
91. De Jong, H. K., van der Poll, T., Wiersinga, W. J. (2010) The systemic pro-inflammatory response in sepsis. *J. Innate Immun.* **2,** 422–430.
92. Guo, Y., Li, J., Hagstrom, E., Ny, T. (2008) Protective effects of plasmin(ogen) in a mouse model of *Staphylococcus aureus*-induced arthritis. *Arthritis Rheum.* **58,** 764–772.
93. Guo, Y., Li, J., Hagstrom, E., Ny, T. (2011) Beneficial and detrimental effects of plasmin(ogen) during infection and sepsis in mice. *PLoS One* **6,** e24774.
94. Li, J., Guo, Y., Holmdahl, R., Ny, T. (2005) Contrasting roles of plasminogen deficiency in different rheumatoid arthritis models. *Arthritis Rheum.* **52,** 2541–2548.
95. Li, J., Ny, A., Leonardsson, G., Nandakumar, K. S., Holmdahl, R., Ny, T. (2005) The plasminogen activator/plasmin system is essential for development of the joint inflammatory phase of collagen type II-induced arthritis. *Am. J. Pathol.* **166,** 783–792.
96. Hamilton, J. A. (2008) Plasminogen activator/plasmin system in arthritis and inflammation: friend or foe? *Arthritis Rheum.* **58,** 645–648.
97. Renckens, R., Weijer, S., de Vos, A. F., Pater, J. M., Meijers, J. C., Hack, C. E., Levi, M., van der Poll, T. (2004) Inhibition of plasmin activity by tranexamic acid does not influence inflammatory pathways during human endotoxemia. *Arterioscler. Thromb. Vasc. Biol.* **24,** 483–488.
98. Min, H. Y., Semnani, R., Mizukami, I. F., Watt, K., Todd III, R. F., Liu, D. Y. (1992) cDNA for Mo3, a monocyte activation antigen, encodes the human receptor for urokinase plasminogen activator. *J. Immunol.* **148,** 3636–3642.
99. Andersson, L., Nilsoon, I. M., Colleen, S., Granstrand, B., Melander, B. (1968) Role of urokinase and tissue activator in sustaining bleeding and the management thereof with EACA and AMCA. *Ann. N. Y. Acad. Sci.* **146,** 642–658.
100. Fiechtner, B. K., Nuttall, G. A., Johnson, M. E., Dong, Y., Sujirattanawimol, N., Oliver, W. C., Jr., Sarpal, R. S., Oyen, L. J., Ereth, M. H. (2001) Plasma tranexamic acid concentrations during cardiopulmonary bypass. *Anesth. Analg.* **92,** 1131–1136.
101. Mojcik, C. F., Levy, J. H. (2001) Aprotinin and the systemic inflammatory response after cardiopulmonary bypass. *Ann. Thorac. Surg.* **71,** 745–754.
102. Wygrecka, M., Jablonska, E., Guenther, A., Preissner, K. T., Markart, P. (2008) Current view on alveolar coagulation and fibrinolysis in acute inflammatory and chronic interstitial lung diseases. *Thromb. Haemost.* **99,** 494–501.
103. Swaisgood, C. M., Aronica, M. A., Swaidani, S., Plow, E. F. (2007) Plasminogen is an important regulator in the pathogenesis of a murine model of asthma. *Am. J. Respir. Crit. Care Med.* **176,** 333–342.
104. Leclercq, A., Houard, X., Loyau, S., Philippe, M., Sebbag, U., Meilhac, O., Michel, J. B. (2007) Topology of protease activities reflects atherothrombotic plaque complexity. *Atherosclerosis* **191,** 1–10.
105. Brands-Nijenhuis, A. V., van Geel, P. P., Meijer, K. (2009) Acute myocardial ischemia in a patient with heterozygous α-2-plasmin inhibitor deficiency. *Blood Coagul. Fibrinolysis* **20,** 599–600.
106. Fay, W. P., Garg, N., Sunkar, M. (2007) Vascular functions of the plasminogen activation system. *Arterioscler. Thromb. Vasc. Biol.* **27,** 1231–1237.
107. Cozen, A. E., Moriwaki, H., Kremen, M., DeYoung, M. B., Dichek, H. L., Slezicki, K. I., Young, S. G., Veniant, M., Dichek, D. A. (2004) Macrophage-targeted overexpression of urokinase causes accelerated atherosclerosis, coronary artery occlusions, and premature death. *Circulation* **109,** 2129–2135.
108. Kuliopulos, A., Covic, L., Seeley, S. K., Sheridan, P. J., Helin, J., Costello, C. E. (1999) Plasmin desensitization of the PAR1 thrombin receptor: kinetics, sites of truncation, and implications for thrombolytic therapy. *Biochemistry* **38,** 4572–4585.

KEY WORDS:
inflammation · serine protease · cell activation · signaling · antigen-presenting cells · endothelial cells · platelets