bjh review

# Molecular mechanisms of fibrinolysis

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## Summary

The molecular mechanisms that finely co-ordinate fibrin formation and fibrinolysis are now well defined. The structure and function of all major fibrinolytic proteins, which include serine proteases, their inhibitors, activators and receptors, have been characterized. Measurements of real time, dynamic molecular interactions during fibrinolysis of whole blood clots can now be carried out *in vitro*. The development of gene-targeted mice deficient in one or more fibrinolytic protein(s) has demonstrated expected and unexpected roles for these proteins in both intravascular and extravascular settings. In addition, genetic analysis of human deficiency syndromes has revealed specific mutations that result in human disorders that are reflective of either fibrinolytic deficiency or excess. Elucidation of the fine control of fibrinolysis under different physiological and pathological haemostatic states will undoubtedly lead to novel therapeutic interventions. Here, we review the fundamental features of intravascular plasmin generation, and consider the major clinical syndromes resulting from abnormalities in fibrinolysis.

**Keywords:** Plasminogen, plasminogen activators, plasminogen activator inhibitor-1, annexin 2, thrombin-activatable fibrinolysis inhibitor.

## Basic concepts of fibrinolysis

Under physiological conditions, both coagulation and fibrinolysis are precisely regulated by the measured participation of substrates, activators, inhibitors, cofactors and receptors. Molecular links between these systems permit localized, timely removal of ongoing or acutely induced fibrin deposits (Table I). These co-ordinated molecular events insure blood fluidity while preventing blood loss (Esmon *et al*, 1999; Degen, 2001; Hajjar, 2003a; Kolev & Machovich, 2003).

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Activation of coagulation ultimately generates thrombin, which results in thrombus formation by conversion of fibrinogen to fibrin and by platelet activation. Plasmin is the major fibrinolytic protease (Fig 1). Plasminogen (PLG), a circulating plasma zymogen, can be converted to plasmin by both tissue PLG activator (tPA) as well as by urokinase (uPA). Through a positive feedback mechanism, plasmin cleaves both tPA and uPA, transforming them from single chain to more active two-chain polypeptides. Fibrin, the major plasmin substrate, regulates its own degradation by binding both PLG and tPA on its surface, thereby localizing and enhancing plasmin generation. While tPA is a weak activator of PLG in the absence of fibrin, its catalytic efficiency for PLG activation is enhanced by at least two orders of magnitude in the presence of fibrin. The affinity between tPA and PLG is low in the absence of fibrin, but increases significantly in its presence.

Once formed, plasmin cleaves fibrin, generating soluble degradation products, and exposing carboxy-terminal lysine (Lys) residues (Fig 2). 'Kringles' 2 of tPA and 1 and 4 of PLG contain lysine-binding sites, which mediate further binding to fibrin, leading to enhanced plasmin generation and fibrin removal. Binding can be blocked by Lys analogues, such as epsilon aminocaproic acid and tranexamic acid, as well as by the recently characterized, thrombin-activatable fibrinolysis inhibitor (TAFI). When activated by thrombin, TAFI removes carboxy-terminal Lys residues, thereby attenuating plasmin generation, stabilizing fibrin thrombi, and establishing a regulatory connection between coagulation and fibrinolysis. Fibrin dissolution is also regulated by inhibitors of PLG activation, such as PLG activator inhibitor-1 (PAI-1), and by inhibitors of plasmin itself, such as $\alpha_2$-plasmin inhibitor ($\alpha_2$-PI). In addition, plasmin bound to fibrin is protected from $\alpha_2$-PI, due to occupancy of its lysine-binding sites. TAFI, on the other hand, decreases this protection by deleting plasmin-binding Lys residues on fibrin.

In addition, diverse cell types promote plasmin generation through their expression of cell surface receptors (Hajjar, 2003b). Endothelial cells, monocytes, macrophages, neutrophils and some tumour cells, all bind PLG, as well as tPA and/or uPA. Their receptors localize cell surface fibrinolytic activity, serve as cofactors in acute or ongoing plasmin generation, and provide specialized environments that are protected from circulating inhibitors.

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**Table I.** Components of the fibrinolytic system.

| |
|---|
| *Zymogen* |
| Plasminogen (N-terminal glutamic acid and lysine variants) |
| *Plasminogen activators* |
| Tissue plasminogen activator (tPA) |
| Urokinase (uPA) |
| *Inhibitors* |
| Plasmin inhibitors |
| $\alpha_2$-plasmin inhibitor ($\alpha_2$-PI) |
| $\alpha_2$-macroglobulin ($\alpha_2$-MG) |
| Protease nexin |
| Plasminogen activator inhibitors |
| Plasminogen activator inhibitor-1 and -2 (PAI-1, PAI-2) |
| $C_1$-esterase inhibitor |
| Protease nexin |
| *Attenuator* |
| Thrombin-activatable fibrinolysis inhibitor (TAFI) |
| *Major receptors* |
| Activating |
| Annexin 2 |
| $\alpha_M\beta_2$ integrin |
| Urokinase receptor (uPAR) |
| Clearance |
| Low-density lipoprotein receptor-related protein (LRP) |
| Mannose receptor |

## Components of the fibrinolytic system

### *Plasminogen*

Synthesized primarily in the liver (Raum *et al*, 1980), PLG, a $M_r$ *c.* 92 000 single-chain proenzyme, circulates in plasma at a concentration of *c.* 1·5 μmol/l, with a half-life of about 2 d (Hajjar, 2003b). Its 791 amino acids are cross-linked by 24 disulphide bridges, 16 of which give rise to five homologous triple loop structures called 'kringles' (Forsgren *et al*, 1987). The first (K1) and fourth (K4) of these 80 amino acid structures impart high and low affinity Lys binding respectively (Miles *et al*, 1988). The lysine-binding domains of PLG mediate its specific interactions with fibrin, cell surface receptors and other proteins, including its circulating inhibitor, $\alpha_2$-PI (Hajjar *et al*, 1986; Miles & Plow, 1991). Post-translational modification of PLG results in two glycosylation variants (forms 1 and 2). The carbohydrate portion of PLG regulates its affinity for cellular receptors, and may also specify its physiological degradation pathway (Hajjar, 2003b).

Activation of PLG, by cleavage of a single Arg-Val peptide bond at position 560–561 (Holvoet *et al*, 1985), gives rise to the active protease, plasmin (Table I). Plasmin contains a typical serine protease catalytic triad (His 602, Asp 645 and Ser 740), and exhibits substrate specificity not limited to fibrin (Saksela, 1985). The circulating form of PLG, amino-terminal glutamic acid (Glu) PLG, is readily converted by limited proteolysis to several modified forms, known collectively as amino-terminal lysine (Lys) PLG (Wallen & Wiman, 1970, 1972). Hydrolysis of the Lys77-Lys78 peptide bond gives rise to a conformationally modified form of the zymogen that more readily binds fibrin, displays two- to threefold higher avidity for cellular receptors, and is activated 10–20 times more rapidly than Glu-PLG (Markus *et al*, 1979; Hoylaerts *et al*, 1982; Holvoet *et al*, 1985). Lys-PLG does not normally

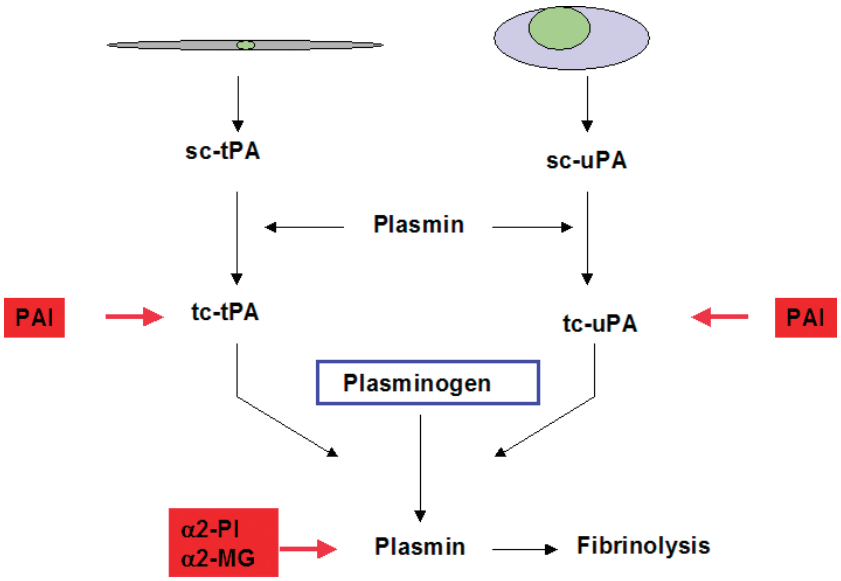


**Fig 1.** Overview of the fibrinolytic system. The zymogen plasminogen is converted to the active serine protease, plasmin, through the action primarily of two-chain tissue plasminogen activator (tc-tPA) or two-chain urokinase (tc-uPA). These activators are secreted as single-chain (sc-tPA and sc-uPA) forms from endothelial cells, and from renal epithelium, monocyte/macrophages, or endothelial cells respectively. Both tPA and uPA can be inhibited by plasminogen activator inhibitor-1 (PAI), while plasmin is inhibited by its major inhibitor, $\alpha_2$-plasmin inhibitor ($\alpha_2$-PI), and to a lesser extent by $\alpha_2$-macroglobulin ($\alpha_2$-MG). Once plasmin is generated, it converts single chain tPA and uPA to double chain forms. It is then rapidly inhibited unless it remains bound to fibrin or to its cell surface receptors. Inhibitors are indicated by red boxes.


 

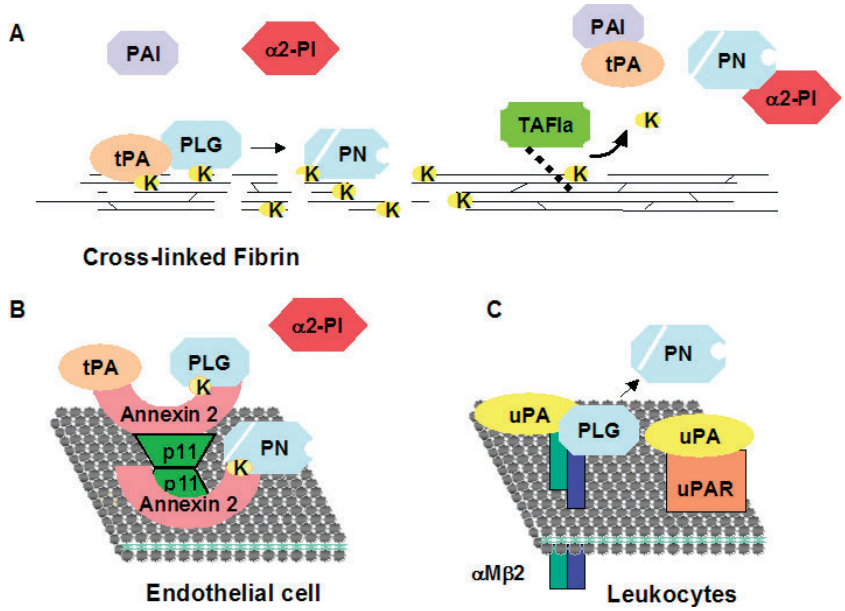


**Fig 2.** Fibrin- and receptor-enhanced plasmin generation. (A) Tissue plasminogen activator (tPA), and plasminogen (PLG), bind fibrin through lysine residues (K). This trimolecular assembly greatly enhances plasmin (PN) generation, which results in further exposure of carboxy-terminal lysines and, ultimately, in fibrin degradation. Fibrin-associated plasmin and tPA are protected from their major inhibitors, $\alpha_2$-plasmin inhibitor ($\alpha_2$-PI) and plasminogen activator inhibitor-1 (PAI) respectively. Thrombin-activatable fibrinolysis inhibitor (TAFIa), a plasma carboxypeptidase, cleaves lysine residues and attenuates fibrin dissolution by decreasing the fibrin-binding sites (K) for fibrinolytic enzymes. Urokinase (uPA) acts independently of fibrin. (B) Annexin 2 is present on the endothelial cell surface as a heterotetramer with the S100 family protein p11. Annexin 2 binds both PLG and tPA, serving as a cofactor for plasmin generation, and protecting plasmin from circulating inhibitors, such as $\alpha_2$-PI. (C) Integrin $\alpha_M\beta_2$ on leucocytes binds both PLG and uPA, serving as a cofactor for plasmin generation. uPA receptor (uPAR) may also bind uPA.

circulate in plasma (Holvoet *et al*, 1985), but has been identified on cell surfaces (Hajjar, 2003b).

Spanning 52·5 kb of DNA on chromosome 6q26, the PLG gene consists of 19 exons (Murray *et al*, 1987; Petersen *et al*, 1990). The gene is closely linked and structurally related to that of apolipoprotein(a), an apoprotein associated with the highly atherogenic low-density lipoprotein (LDL)-like particle lipoprotein(a) (McLean *et al*, 1987). Apolipoprotein(a) and PLG are more distantly related to other kringle-containing proteins such as tPA, uPA, hepatocyte growth factor and macrophage-stimulating protein (Nakamura *et al*, 1989; Ichinose, 1992). The significance of the latter two proteins to fibrinolysis remains to be determined.

## *Characterization of plasmin(ogen) function*

The diverse physiological roles of plasmin have become evident with the development of gene-targeted PLG-deficient mice (Table II). These mice undergo normal embryogenesis and development, are fertile, and survive to adulthood (Bugge *et al*, 1995a). However, in addition to runting and ligneous conjunctivitis (Drew *et al*, 1998), they display a predisposition to vascular occlusion with spontaneous thrombi appearing in the liver, stomach, colon, rectum, lung and pancreas. Fibrin deposition is seen in the liver, and ulcerative lesions in the gastrointestinal tract and rectum. These results suggest that PLG is not strictly required for normal development, but is essential for maintenance of postnatal fibrin homeostasis in both intra- and extra-vascular settings.

## *Plasminogen activators*

*Tissue plasminogen activator.* One of two major endogenous PLG activators, $M_r$ *c.* 72 000 tPA consists of 527 amino acids (Pennica *et al*, 1983; Fig 1). This glycoprotein contains five structural domains, including a fibronectin-like 'finger', an epidermal growth factor-like cassette, two 'kringle' structures homologous to those of PLG and a serine protease domain. Cleavage of the Arg275-Ile276 peptide bond by plasmin converts tPA to a disulphide-linked, two-chain form (Pennica *et al*, 1983). While single-chain tPA is less active than two-chain tPA in the fluid phase, both forms demonstrate equivalent activity when fibrin bound (Tate *et al*, 1987).

The two glycosylation forms of tPA are distinguishable by the presence (type 1) or absence (type 2) of a complex N-linked oligosaccharide moiety on Asn184 (Hajjar, 2003b). Both types contain high mannose carbohydrate on Asn117, complex oligosaccharide on Asn448 and an O-linked α-fucose residue on Thr61 (Harris *et al*, 1991). The carbohydrate moieties of tPA may modulate its functional activity, regulate its binding to cell surface receptors and specify degradation pathways.

Located on chromosome 8q11 (Ny *et al*, 1984), the gene for human tPA consists of 14 exons spanning a total of 36·6 kb (Degen *et al*, 1986). *In vitro*, many agents exert small effects on

**Table II.** Some gene deletion mouse models relevant to intravascular fibrinolysis.

| Genotype* | Phenotype |
|---|---|
| *Plasminogen* | |
| $PLG^{-/-}$ | Spontaneous thrombosis, runting, premature death |
| | Fibrin in liver, lungs, stomach; gastric ulcers |
| | Impaired wound healing; ligneous conjunctivitis |
| | Impaired monocyte recruitment |
| | Impaired neointima formation after electrical injury |
| | Impaired dissemination of *Borrelia burgdorferi* |
| | Reduced excitotoxic neuronal cell death in brain |
| *Plasminogen activators* | |
| $tPA^{-/-}$ | Reduced lysis of fibrin clot |
| | Increased endotoxin-induced thrombosis |
| $uPA^{-/-}$ | Occasional fibrin in liver/intestine |
| | Rectal prolapse, ulcers of eyelids, face, ears |
| | Reduced macrophage degradation of fibrin |
| | Increased endotoxin-induced thrombosis |
| $uPA^{-/-}/tPA^{-/-}$ | Reduced growth, fertility and lifespan; cachexia |
| | Fibrin deposits in liver, gonads, lungs |
| | Ulcers in intestine, skin, ears; rectal prolapse |
| | Impaired clot lysis |
| *Inhibitors* | |
| $PAI\text{-}1^{-/-}$ | Mildly increased lysis of fibrin clot |
| | Resistance to endotoxin-induced thrombosis |
| $LRP^{-/-}$ | Embryonic lethal day 13·5 postconception |
| $TAFI^{-/-}$ | Essentially normal |
| *Receptors* | |
| $uPAR^{-/-}$ | Essentially normal |
| | Reduced macrophage PLG activation *in vitro* |
| | Normal matrix degradation |
| Annexin $2^{-/-}$ | Mild runting, fibrin deposition in microvasculature |
| | Impaired clearance of arterial thrombi |
| | Impaired postnatal neoangiogenesis |

PLG, plasminogen; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor-1; LRP, low-density lipoprotein receptor-related protein; TAFI, thrombin-activatable fibrinolysis inhibitor; uPAR, urokinase plasminogen activator receptor.

*Several examples of combined gene deletion mouse models exist, which are beyond the scope of this review.

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the expression of tPA mRNA, but relatively few enhance tPA synthesis without also augmenting PAI-1 synthesis. Agents that regulate tPA gene expression independently of PAI-1 include histamine, butyrate, retinoids, arterial levels of shear stress and dexamethasone. Forskolin, which increases intracellular cyclic adenosine 3,5-monophosphate (cAMP) levels, has been reported to decrease synthesis of both tPA and PAI-1 (Hajjar, 2003b).

The tPA is synthesized and secreted primarily by endothelial cells. However, expression of tPA appears to be restricted to 7–30 nm diameter precapillary arterioles, postcapillary venules and vasa vasora; much less expression is seen in endothelial cells of the femoral artery, femoral vein, carotid artery or aorta (Levin & del Zoppo, 1994). In the mouse lung, bronchial artery endothelial cells express tPA antigen, especially at branch points, while pulmonary blood vessels are generally negative. The release of tPA is governed by a variety of stimuli, such as thrombin, histamine, bradykinin, adrenaline, acetylcholine, Arg vasopressin, gonadotropins, exercise, venous occlusion and shear stress, its circulating half-life is exceptionally short (*c.* 5 min). Although expressed by extravascular cells, tPA appears to be the major intravascular activator of PLG (Hajjar, 2003a).

*Urokinase.* A second endogenous PLG activator, single-chain uPA or prourokinase, is a $M_r$ *c.* 54 000 glycoprotein consisting of 411 amino acids (Table I). uPA possesses an epidermal growth factor-like domain, a single PLG-like kringle and a classical serine protease catalytic triad (His204, Asp255, Ser356; Kasai *et al*, 1985). Cleavage of the Lys158-Ile159 peptide bond by plasmin or kallikrein converts single-chain uPA to a disulphide-linked two-chain derivative. Located on chromosome 10q26, the human uPA gene is encoded by 11 exons spanning 6·4 kb, and is expressed by endothelial cells, macrophages, renal epithelial cells and some tumour cells (Holmes *et al*, 1985; Riccio *et al*, 1985).

Two-chain uPA occurs in both high ($M_r$: *c.* 54 000) and low-molecular weight ($M_r$: *c.* 33 000) forms that differ by the presence or absence, respectively, of a 135-residue amino-terminal fragment released by plasmin cleavage between Lys135 and Lys136. Although both forms are capable of activating PLG, only the high-molecular weight form binds to the uPA receptor (uPAR; Hajjar, 2003a). uPA has much lower affinity for fibrin than tPA, and is an effective PLG activator in both the presence and the absence of fibrin (Gurewich *et al*, 1984; Lijnen *et al*, 1986). Interestingly, activation of Glu-PLG by two-chain uPA is increased in the presence of fibrin by about 10-fold, even though uPA does not bind to fibrin. This may reflect a conformational change in PLG upon binding to fibrin. In contrast, single-chain uPA has considerable fibrin specificity, yet an intrinsic PLG activating capacity of <1% of that observed for two-chain uPA (Hajjar, 2003b).

*Accessory activators and fibrinolysins.* Under certain conditions, proteases that are traditionally classified within the intrinsic arm of the coagulation cascade have been shown to activate PLG directly. These include kallikrein, factor XIa and factor XIIa (Colman, 1968; Goldsmith *et al*, 1978), but normally account for no more than 15% of total plasmin-generating activity in plasma (Hajjar, 2003b). In addition, the membrane type 1 matrix metalloproteinase (MT1-MMP) appears to exert fibrinolytic activity in the absence of PLG, and may explain the unexpectedly mild phenotype observed in PLG-deficient mice (Hiraoka *et al*, 1998). Factor VII-activating protease has also been reported to serve as an *in vivo* activator of single-chain PLG activators, but its *in vivo* role remains to be determined (Romisch *et al*, 1999).

*Physiological function of plasminogen activators.* Because there are no published clinical examples of complete deficiency of tPA or uPA in humans, the most compelling studies of the physiological functions of PLG activators derive from gene disruption in mice (Carmeliet *et al*, 1994; Table II). Both uPA and tPA null mice exhibit normal fertility and embryonic development. However, $uPA^{-/-}$ mice developed rectal prolapse, non-healing ulcerations of the face and eyelids, and occasional fibrin deposition in tissues; endotoxin-induced thrombosis is significantly enhanced, although rates of pulmonary clot lysis are normal. tPA-deficient mice, on the other hand, have a normal spontaneous phenotype, but have less efficient lysis of artificially induced pulmonary thrombi, as well as enhanced thrombus formation in response to endotoxin. Doubly deficient ($tPA^{-/-}$; $uPA^{-/-}$) mice exhibit rectal prolapse, non-healing ulceration, runting and cachexia, with extensive fibrin deposition in the liver, intestine, gonads and lung. Not surprisingly, clot lysis is markedly impaired. These findings demonstrate that tPA and uPA are not essential for normal embryologic development, but are crucial to thrombolysis and fibrinolytic surveillance in the adult.

## Inhibitors and attenuators of fibrinolysis

### *Plasmin inhibitors*

The action of plasmin is negatively modulated by a family of serine protease inhibitors known as the serpins (Travis & Salvesan, 1983; Fig 1). All serpins form an irreversible complex with the active site serine of the target protease, following proteolytic cleavage of the inhibitor by the target protease. Both protease and inhibitor lose their activity.

A single-chain glycoprotein of $M_r$ *c.* 70 000, $\alpha_2$-PI, also known as $\alpha_2$-antiplasmin, circulates in plasma at high concentration (*c.* 0·9 nmol/l) with a plasma half-life of about 2·4 d. $\alpha_2$-PI contains about 13% carbohydrate by mass, and consists of 452 amino acids with two disulphide bridges (Holmes *et al*, 1987). In humans, the gene is located on chromosome 18q21–22, and contains 10 exons distributed over 16 kb of DNA (Hirosawa *et al*, 1988). The promoter region of the $\alpha_2$-PI gene contains a hepatitis B virus-like enhancer element that directs tissue-specific expression in the liver (Holmes *et al*, 1987). $\alpha_2$-PI is also a constituent of platelet α-granules (Plow & Collen, 1981). Thus, plasmin released into flowing blood or in the vicinity of a platelet-rich thrombus, is immediately neutralized by $\alpha_2$-PI. $\alpha_2$-PI is cleaved at its Arg364-Met365 peptide bond, and then forms a lysine-binding site-dependent $\alpha_2$-PI–plasmin complex, which is cleared in the liver.

Several additional proteins can act as plasmin or PAIs (Table I). $\alpha_2$-Macroglobulin is a $M_r$ 725 000 dimeric protein synthesized by endothelial cells and macrophages, and found in platelet α-granules. This non-serpin forms non-covalent complexes with plasmin, and inhibits its activity with *c.* 10% of the efficiency exhibited by $\alpha_2$-PI (Aoki *et al*, 1978a). $C_1$-esterase inhibitor may serve as an inhibitor of tPA in plasma (Huisman *et al*, 1995). Protease nexin may function as a non-circulating cell surface inhibitor of trypsin, thrombin, factor Xa, urokinase or plasmin (Scott *et al*, 1985), resulting in protease–inhibitor complexes that are endocytosed via a specific nexin receptor (Hajjar, 2003a).

### *Plasminogen activator inhibitors*

*Plasminogen activator inhibitor-1.* The most ubiquitous of the two major PAIs is PAI-1 (Table I). This $M_r$ *c.* 52 000 single chain, cysteine (Cys)-less glycoprotein is released by endothelial cells, monocytes, macrophages, hepatocytes, adipocytes and platelets (Ny *et al*, 1986; Samad *et al*, 1996). Release of PAI-1 is stimulated by many cytokines, growth factors and lipoproteins common to the global inflammatory response (Hajjar, 2003b). The PAI-1 gene consists of nine exons, spanning 12·2 kb on chromosome 7q21 (Loskutoff *et al*, 1987). Activity of this labile serpin is stabilized by complex formation with vitronectin, a component of both plasma and pericellular matrix (Declerck *et al*, 1988; Mottonen *et al*, 1992). The upstream regulatory region of the human PAI-1 gene contains a strong endothelial cell/fibroblast-specific element, a glucocorticoid-responsive enhancer and transforming growth factor (TGF)-β responsive elements (Hajjar, 2003b). Agents that have been shown to enhance expression of PAI-1 at the message level, the protein level, or both, without affecting tPA synthesis, include the inflammatory cytokines lipopolysaccharide, interleukin-1, tumour necrosis factor-α, TGF-β, basic fibroblast growth factor (bFGF), very LDL, lipoprotein(a), angiotensin II, thrombin and phorbol esters (Etingin *et al*, 1990; Stiko-Rahm *et al*, 1990; Hajjar, 2003b).

The PAI-1 is the most important and most rapidly acting physiological inhibitor of both tPA and uPA. Transgenic mice that overexpress PAI-1 exhibit thrombotic occlusion of tail veins and swelling of hind limbs within 2 weeks of birth (Erickson *et al*, 1990). Mice deficient in PAI-1, on the other hand, exhibit normal fertility, viability, tissue histology and development, and show no evidence of haemorrhage (Carmeliet *et al*, 1993a). These observations contrast with the moderately severe bleeding disorder observed in a human patient with complete PAI-1 deficiency (Fay *et al*, 1992).

*Plasminogen activator inhibitor-2.* Plasminogen activator inhibitor-2, a 393 amino acid member of the serpin family, was originally purified from human placenta (Ye *et al*, 1987; Table I). The gene is located on chromosome 18q21, spans 16·5 kb, and contains eight exons (Ye *et al*, 1989). PAI-2 exists as both a $M_r$ *c.* 47 000 non-glycosylated intracellular form and a $M_r$ *c.* 60 000 glycosylated form, secreted by leucocytes and fibrosarcoma cells. Functionally, PAI-2 inhibits both two-chain tPA and two-chain uPA with comparable efficiency, but it is less effective towards single-chain tPA, and does not inhibit prourokinase. Significant levels of PAI-2 are found in human plasma only during pregnancy.

### *Thrombin-activatable fibrinolysis inhibitor*

The single-chain plasma glycoprotein, TAFI, is also known as procarboxypeptidase B (Eaton *et al*, 1991), procarboxypeptidase U (unstable) and procarboxypeptidase R (for Arg). This $M_r$ *c.* 60 000 polypeptide circulates in plasma at concentrations of *c.* 100 nmol/l (6 μg/ml; Nesheim, 2003). Its gene, located on chromosome 13q14, spans 11 exons. Protein levels frequently correlate with polymorphisms in the promoter and 3′-untranslated region (Bajzar *et al*, 1995). TAFI is expressed by the liver and is present in platelets (Nesheim, 2003). Its activation by thrombin is accelerated in the presence of thrombomodulin by about 1250-fold. At high concentrations, plasmin can also activate TAFI. Activated TAFI (TAFIa) is a $M_r$ *c.* 35 000, Zn-dependent carboxypeptidase with specificity for carboxy-terminal Arg and Lys residues. Because these residues are binding sites for PLG and for tPA on fibrin and on annexin 2 (Redlitz *et al*, 1995), TAFIa functions as a potent attenuator of fibrinolysis. Unstable at body temperature, circulating TAFI has a half-life of about 8 min. Certain polymorphisms enhance its half-life, resulting in greater clot protection from fibrinolysis (Nesheim, 2003). TAFI null mice, however, have an essentially normal baseline phenotype (Nagashima *et al*, 2002).

Attenuation of thrombin generation by the natural anticoagulant, activated protein C (APC), also decreases TAFI activation, thus explaining APCs reported profibrinolytic effect. In the same manner, reduction of TAFIa, either by direct inhibition or by blockade of intrinsic pathway generation of thrombin, results in enhanced endogenous clot lysis in a rabbit jugular vein model of thrombolysis (Minnema *et al*, 1998). On the contrary, the presence of the factor V Leiden mutation, which confers resistance to APC, leads to enhanced thrombin generation, increased TAFI activation and blockade of fibrinolysis, an additional risk factor for thrombosis (Antovic & Blomback, 2002). Thus, the coagulation and fibrinolytic pathways are closely linked.

## *Cellular receptors*

Although structurally diverse, cell surface fibrinolytic receptors can be classified into two groups whose integrated actions are likely to be essential for homeostatic control of plasmin activity (Hajjar, 1995; Table I). 'Activation' receptors localize and potentiate PLG activation, while 'clearance' receptors eliminate plasmin and PLG activators from the blood or focal microenvironments.

### *Activation receptors*

*Plasminogen receptors.* Plasminogen receptors are a diverse group of proteins expressed on a wide array of cell types (Hajjar, 1995). Reported receptors include α-enolase, glycoprotein IIb/IIIa complex, the Heymann nephritis antigen, amphoterin, integrin $\alpha_M\beta_2$ and annexin 2, which are expressed primarily on monocytoid cells, platelets, renal epithelial cells, neuroblastoma cells, leucocytes and endothelial cells respectively (Hajjar, 1995). These binding proteins commonly interact with the kringle structures of PLG through carboxyl-terminal Lys residues (Miles *et al*, 1991).

*The urokinase receptor.* The uPAR is expressed on monocytes, macrophages, fibroblasts, endothelial cells and a variety of tumour cells (Hajjar, 1995; Table I). The uPAR cDNA, which was cloned and sequenced from a human fibroblast cDNA library (Roldan *et al*, 1990), encodes a protein of 313 amino acids with a 21-residue signal peptide. The gene consists of seven exons distributed over 23 kb of genomic DNA, and places this glycoprotein within the Ly-1/elapid venom toxin superfamily of Cys-rich proteins (Behrendt *et al*, 1990; Casey *et al*, 1994). uPAR is anchored to the plasma membrane through glycosylphosphatidylinositol linkages (Ploug *et al*, 1991). uPAR-bound uPA maintains its activity and susceptibility to the physiological inhibitor, PAI-1. Formation of the uPA–PAI-1 complex appears to hasten clearance of uPA by hepatic or monocytoid cells (Hajjar, 1995). uPAR appears to regulate cellular signalling and adhesion (Chapman, 1997). uPAR binds the adhesive glycoprotein vitronectin at a site distinct from the uPA-binding domain (Waltz & Chapman, 1994; Wei *et al*, 1994), and uPAR-transfected renal epithelial cells acquire enhanced adhesion to vitronectin, while they lose their adhesion to fibronectin (Wei *et al*, 1996). uPAR, furthermore, co-localizes with integrins in focal contacts and at the leading edge of migrating cells (Xue *et al*, 1994), and also associates with caveolin, a major component of caveolae, structures abundant in endothelial cells and thought to participate in signalling events (Anderson, 1993; Stahl & Mueller, 1995; Okamoto *et al*, 1998). Thus, uPAR appears to integrate cellular adhesion with proteolysis. uPAR deficiency in mice is associated with normal fertility, development and haemostasis (Bugge *et al*, 1995b).

*Integrin* $\alpha_M\beta_2$. Neutrophils express integrin $\alpha_M\beta_2$, and also release uPA, a $\alpha_M\beta_2$ ligand. $\alpha_M\beta_2$ binds both uPA ($K_d$: *c.* 40 nmol/l) and PLG ($K_d$: *c.* 1 nmol/l) in a dose-dependent, saturable manner. This results in a 50-fold acceleration of plasmin generation, contributing to the degradation of fibrin-rich thrombi (Pluskota *et al*, 2004).

*Annexin 2.* A member of the annexin superfamily of calcium-dependent, phospholipid-binding proteins, annexin 2 is highly conserved and abundantly expressed on endothelial cells, monocyte/macrophages, myeloid cells, developing neuronal cells and some tumour cells (Hajjar & Krishnan, 1999). All annexin family members have in common a large conserved C-terminal 'core' region, and a smaller, more variable N-terminal 'tail'. The human annexin 2 gene consists of 13 exons distributed over 40 kb of genomic DNA on chromosome 15q21 (Spano *et al*, 1990).

Annexin 2 possesses binding affinity for both PLG ($K_d$: *c.* 114 nmol/l) and tPA ($K_d$: *c.* 30 nmol/l), but not uPA (Hajjar & Hamel, 1990; Hajjar, 1991). In a purified-protein system, native human placental annexin 2 stimulates the catalytic efficiency of tPA-dependent PLG activation by 60-fold (Cesarman *et al*, 1994). This effect is completely inhibited in the presence of Lys analogues or upon treatment of annexin 2 with the TAFI-like enzyme carboxypeptidase B, which removes basic carboxyl-terminal amino acids. Although it lacks a classical signal peptide, annexin 2 is translocated to the endothelial cell surface through a mechanism that requires phosphorylation of tyrosine 23 and the accessory protein, p11 (Deora *et al*, 2004). The annexin 2 heterotetramer, which consists of two annexin monomers and two p11 subunits, may have even greater stimulatory effects on tPA-dependent plasmin generation (Kassam *et al*, 1998).

Plasminogen and tPA bind to distinct annexin 2 domains. Lys307 appears to be crucial for the effective interaction of PLG with annexin 2 (Hajjar *et al*, 1994). The atherogenic LDL-like particle, lipoprotein(a), competes with PLG for binding to annexin 2 *in vitro* (Hajjar *et al*, 1989), reducing cell surface plasmin generation. tPA binding to annexin 2 requires residues 8–13 (Leu-Cys-Lys-Leu-Ser-Leu) within the receptor's amino-terminal tail domain (Hajjar *et al*, 1998). This sequence is a target for homocysteine (HC), a thiol-containing amino acid that accumulates in association with nutritional deficiencies of vitamin B6, vitamin B12, or folic acid, or in inherited abnormalities of cystathionine β-synthase, methylenetetrahydrofolate reductase, or methionine synthase, and is associated with atherothrombotic disease (Refsum *et al*, 1998). *In vitro*, HC impairs the intrinsic fibrinolytic system of the endothelial cell by *c.* 50% (Hajjar, 1993) by forming a covalent derivative with Cys9, thus preventing its interaction with t-PA (Hajjar *et al*, 1998). The half-maximal dose of HC for inhibition of tPA binding to annexin 2 is *c.* 11 μmol/l HC, a value close to the upper limit of normal for HC in plasma (10 μmol/l).

Several studies suggest a physiological role for annexin 2 in fibrin homeostasis. Mice with total deficiency of annexin 2 display impaired clearance of artificial arterial thrombi, fibrin deposition in the microvasculature and angiogenic defects in a variety of tissues (Ling *et al*, 2004). In rats, arterial thrombosis can be significantly attenuated by pretreatment with intravenous annexin 2 (Ishii *et al*, 2001).

### *Clearance receptors*

Both uPA and tPA are cleared from the circulation via the liver (Bu *et al*, 1994). *In vitro*, clearance of tPA–PAI-1 complexes appears also to be mediated by a large two-chain receptor called the LDL receptor-related protein (LRP; Beiseigel *et al*, 1989; Brown *et al*, 1991). This complex interaction requires both growth factor and finger domains of tPA. An additional $M_r$ *c.* 39 000 'receptor-associated protein' co-purifies with LRP and may regulate the binding and uptake of LRP ligands. Interestingly, LRP 'knock-out' embryos undergo developmental arrest by 13·5 d after conception, suggesting that regulation of serine protease activity is crucial for early embryogenesis (Herz *et al*, 1992). Although several PAI-1-independent clearance pathways for tPA have been proposed involving the large LRP subunit (Bu *et al*, 1992), the mannose receptor (Otter *et al*, 1991) or an α-fucose-specific receptor (Hajjar & Reynolds, 1994), *in vivo* studies in mice suggest that LRP and the mannose receptor play a dominant role in tPA clearance (Narita *et al*, 1995).

## Mouse models of fibrinolysis

Much of what we understand about fibrinolytic function in humans reflects pioneering studies conducted in genetically engineered mouse models (Poplis & Castelino, 2002). The discovery of fibrin deposition in mice deficient in PLG, uPA alone, uPA and tPA together and annexin 2, for example, established a central role for these molecules in regulating baseline fibrin homeostasis. The absence of fibrin deposition in the tPA and uPAR knockouts, on the other hand, suggests that these molecules may be dispensable, at least in the mouse under resting conditions.

Additional investigations have illustrated the far-reaching, and sometimes paradoxical, roles of the fibrinolytic system in diverse physiological processes. For example, while tPA may function in neuronal plasticity and may protect against demyelination following nerve crush injury, it also appears to mediate excitatory neuronal cell death (Strickland, 2000). PLG, similarly, is required for leucocyte recruitment to sites of inflammation, but may also be co-opted from a host organism by bacteria during the invasive phase of infection (Plow & Hoover-Plow, 2004). The complex roles of the fibrinolytic system in cardiovascular disease are illustrated by studies in which (i) postinjury arterial neointima formation required uPA and PLG, but not tPA or uPAR, (ii) atherosclerotic plaque development was accelerated by deficiency of PLG, but not uPA, tPA, or PAI-1 and (iii) loss of uPA, but not tPA, prevented cardiac rupture following experimental myocardial infarction (Carmeliet & Collen, 2000; Knowles & Maeda, 2000). Furthermore, plasmin and its parent molecules appear to be crucial for vascular remodelling and angiogenesis (Lijnen, 2001; Pepper, 2001), while the impact of PAI-1 on experimental intimal hyperplasia seems to depend upon the model system employed (Fay, 2004).

Despite these studies, it is important to note those instances in which the murine fibrinolytic function may depart from that of the human (Lijnen *et al*, 1994). For example, while neither PAI-1 nor $\alpha_2$-PI deficiency in the mouse leads to overt bleeding (Carmeliet *et al*, 1993b; Lijnen *et al*, 1999), the same human deficiencies are associated with a haemorrhagic state (Hajjar, 2003b). Similarly, while ligneous conjunctivitis is the major manifestation of PLG

deficiency in humans, it is seen only in PLG knockout mice with specific genetic backgrounds (Schuster & Seregard, 2003). While elevated plasma TAFI levels in humans may be associated with venous thromboembolism, the phenotype of complete TAFI deficiency in the mouse is normal (Bouma & Meijers, 2003).

## The fibrinolytic actions of plasmin

### *Degradation of fibrin and fibrinogen*

*Fibrinogen.* When plasma antiplasmin activity is overwhelmed, as when PLG activators are used for the treatment of thrombosis, circulating fibrinogen may be degraded by plasmin (Fig 3). Fibrinogen possesses distinct proteolytic cleavage sites for plasmin, which give rise to fragments [Aα, Bβ and fragment fibrinopeptide B (FPB)] from the C- and N-termini of fibrinogen's three polypeptide chains. The resulting $M_r$ *c.* 250 000 molecule is termed fragment X and represents a clottable form of fibrinogen. Additional cleavage events may release other peptides, and in a series of subsequent reactions, plasmin may further cleave the three polypeptide chains that connect the D- and E-domains. Some of these fragments inhibit the spontaneous polymerization of fibrinogen (Hajjar, 2003b).

*Fibrin.* Plasmin degradation of fibrin leads to a distinct set of molecular products (Fig 3; Pizzo *et al*, 1973). When fibrin, cross-linked by factor XIII, is degraded by plasmin, fragments known as D-dimers are released (Hajjar, 2003b). Assays for cross-linked D-dimer fragments are employed clinically to identify disseminated intravascular coagulation-like states associated with excessive plasmin-mediated fibrinolysis. Additional fibrin breakdown products are formed and may have biological activities, including inhibition of platelet function, potentiation of the hypotensive effects of bradykinin, chemotaxis and immune modulation (Hajjar, 2003b).

## The non-fibrinolytic actions of plasmin

A large number of *in vitro* studies suggest a role for plasmin in tissue remodelling, arthritis and toxic neuronal death (Hajjar, 2003b). Basement membrane proteins, such as thrombospondin, laminin, fibronectin and fibrinogen, are readily degraded by plasmin *in vitro*, suggesting possible roles in inflammation, tumour cell invasion, embryogenesis, ovulation, neurodevelopment and prohormone activation *in vivo*. Plasmin can both activate and inactivate coagulation factors V and IX. Plasmin also activates MMPs 1 and 3, thereby facilitating the degradation of matrix proteins, such as the collagens, laminin, fibronectin, vitronectin, elastin, aggrecan and tenascin C. Impaired wound

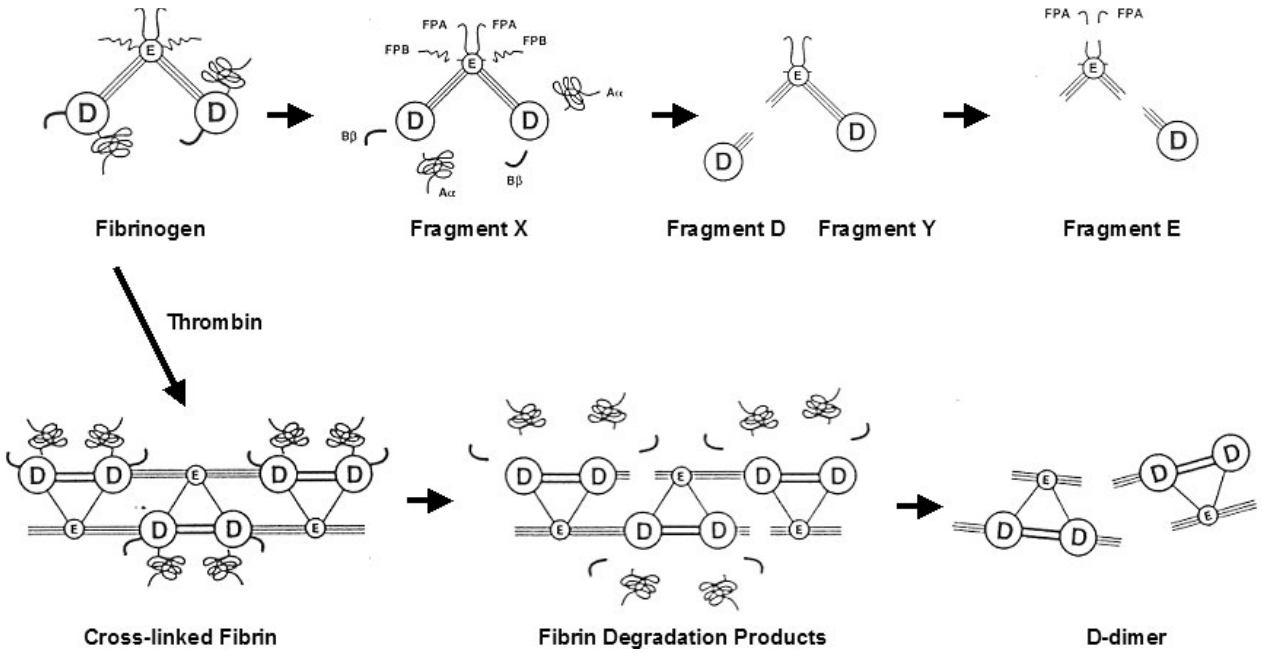


**Fig 3.** Degradation of fibrinogen and cross-linked fibrin by plasmin. (Top panel) Plasmin initially cleaves the C-terminal regions of the α- and β-chains within the D-domain of fibrinogen, releasing the Aα- and Bβ-fragments. In addition, a fragment-containing fibrinopeptide B (FPB) from the N-terminal region of the β-chain is also released, giving rise to the intermediate fragment known as 'fragment X'. Subsequently, plasmin cleaves the three polypeptide chains that connect the D- and E-domains, giving rise to fragments D, Y and E. (Bottom panel) Fibrinogen can also be polymerized by thrombin to form fibrin. When degrading cross-linked fibrin, plasmin initially cleaves the C-terminal region of the α- and β-chains within the D-domain. Subsequently, some of the connecting regions between the D- and E-domains are severed. Fibrin is ultimately solubilized upon hydrolysis of additional peptide bonds within the central portions of the coiled-coil connectors, giving rise to fibrin degradation products such as D-dimer. Adapted from Hajjar (2003b), with permission from Elsevier.

healing is observed in the PLG 'knockout' (Romer *et al*, 1996), which is reversed upon simultaneous deletion of fibrinogen (Table II; Bugge *et al*, 1996; Carmeliet *et al*, 1997; Coleman *et al*, 1997). Interestingly, kainate-induced excitotoxicity and attendant neuronal cell dropout in the hippocampus is not observed in PLG knockout mice but does occur in fibrinogen-deficient animals (Chen & Strickland, 1997).

The role of the fibrinolytic system in vascular remodelling during atherosclerosis is complex (Hajjar *et al*, 2005). Atherogenesis appears to be a response to vascular insult (Ross, 1999). Plasmin may play a role in the activation of growth factors, and in the blood vessel's proliferative response to injury, by converting latent TGF-β to its physiologically relevant active state (Lyons *et al*, 1990). In the evolution of an injury to the endothelial cell lining of blood vessels, deposition of intravascular fibrin and organization of a thrombus occurs. As the injury heals, fibrin participates in plaque growth and luminal narrowing. In the absence of PAI-1, there is less neointima formation and reduced luminal stenosis, possibly due to more rapid resolution of fibrin. In areas of the vasculature where injury is not associated with fibrin deposition, however, absence of PAI-1 may lead to enhanced lesion formation, as cells that invade the developing plaque may require plasmin activity for their directed migration (Lyons *et al*, 1990; Hajjar, 2003b).

### *Angiostatin and related plasminogen fragments*

Angiostatin is a circulating inhibitor of angiogenesis originally isolated from the urine of Lewis lung carcinoma-bearing mice (O'Reilly *et al*, 1995). This $M_r$ *c.* 38 000 polypeptide is identical to kringles 1, 2, 3 and 4 of PLG, and inhibits bFGF-stimulated endothelial cell proliferation *in vitro*, possibly by inducing apoptosis. Angiostatin appears to blocks new blood vessel formation in both the chick chorioallantoic membrane and mouse cornea assays. The cellular target or receptor for angiostatin is unknown although an endothelial cell-binding site distinct from annexin 2 has been proposed. In other studies, kringle 5 of PLG was found to be an even more potent inhibitor of growth factor-stimulated endothelial cell proliferation (Cao *et al*, 1997).

## Developmental regulation and disorders of plasmin generation

### *Developmental regulation of the fibrinolytic system*

In the resting, non-stressed state, the plasmin-generating potential in the newborn is significantly less than that of the adult (Hajjar, 2003b). Although the amino acid composition and apparent molecular mass of neonatal PLG are indistinguishable from those of the adult protein, PLG in the neonate is heavily glycosylated, less readily activated by tPA, and only weakly bound to the endothelial cell surface. Plasma concentrations of PLG in the neonate are *c.* 50% of those observed in adults. In contrast, levels of histidine-rich glycoprotein, a carrier protein that may limit PLG's interaction with fibrin, are reduced by 50–80% in healthy, term newborns (Hajjar, 2003b). tPA antigen and activity levels are also reduced by *c.* 70%, compared with adult values. Stressed infants, such as those with severe congenital heart disease or respiratory distress syndrome, may have tPA antigen levels that are increased by up to eightfold. In contrast, the principal plasmin inhibitors undergo only minimal change from birth to adulthood. Thus, reduced fibrinolytic activity may contribute to the thrombogenic state commonly observed in the newborn, but this predilection may be reversed under conditions of physiological stress (Hajjar, 2003b).

### *Fibrinolytic activity during pregnancy and puerperium*

Pregnancy is a prothrombotic, hypofibrinolytic state. Overall fibrinolytic activity, as reflected in euglobulin lysis activity, is reduced, and increased fibrin deposition is suggested by increasing D-dimer levels throughout pregnancy (Hellgren, 1996). Both PLG and fibrinogen levels in plasma increase by 50–60% in the third trimester, accompanied by an increase in PAI-1 levels to three times their normal level, while PAI-2 levels rise to 25 times their level in early pregnancy. Less dramatic increases in both uPA and tPA levels are also observed. Within 1 h of delivery, both concentrations of both PAI-1 and PAI-2 decrease; they return to normal levels within 3–5 d. In pre-eclampsia, the haemostatic and fibrinolytic imbalances seen in pregnancy are further exaggerated. In this disorder, circulating PAI-1 levels exceed those in normal pregnancy, and fibrin deposition is seen in glomerular capillaries and spiral arteries of the placenta. Interestingly, levels of PAI-2, a marker of placental function, are reduced during pre-eclampsia compared with normal pregnancy, and this decrease correlates with intrauterine growth retardation of the fetus (Hajjar, 2003b).

### *Fibrinolytic deficiency and thrombosis*

Partial human PLG deficiency was first described in a man with a history of repeated episodes of thrombophlebitis, intracranial and mesenteric venous thrombosis, and pulmonary embolism (Aoki *et al*, 1978b). Reduced plasma PLG activity (50% of normal) resulted from an Ala601Thr point mutation. Additional patients with this defect or related substitutions have now been described (Ichinose *et al*, 1991). Acquired PLG deficiency, as may occur in liver disease, sepsis and Argentine haemorrhagic fever because of decreased synthesis and/or increased catabolism, has frequently been associated with thrombotic vascular occlusion.

Congenital PLG deficiency has been classified into two types. In type I, the concentration of immunoreactive PLG is reduced in parallel with functional activity. In a study of consecutive patients with thrombophilia, the prevalence of PLG deficiency was 1·9%. Approximately half of these individuals had other

risk factors, such as deficiency of antithrombin III, protein C, or protein S, or resistance to APC. Among 93 patients with type I heterozygous PLG deficiency, the prevalence of thrombosis was 24%, or 9% when the propositi were excluded (Hajjar, 2003b).

In one well-documented case of type I PLG deficiency, an infant with <1% of normal PLG antigen and activity presented with hydrocephalus, central nervous system malformations, poor wound healing, recurrent respiratory infections and severe ligneous conjunctivitis (fibrinous membrane over the eyes). The latter resolved completely upon infusion of Lys-PLG (Schott *et al*, 1998). This case illustrates the importance of PLG in extravascular fibrinolysis, and underscores the role of PLG deficiency as a relatively weak predisposing risk factor for thrombosis.

In type II PLG deficiency, immunoreactive protein is normal while functional activity is reduced (Ichinose *et al*, 1991). In a study of a Japanese cohort, 94% of 129 families with type II dysplasminogenaemia had the Ala601Thr mutation, while 3% and 1% had Val355Phe and Asp676Asn mutations respectively (Tsutsumi *et al*, 1996). In this study, *c.* 27% of individuals with dysplasminogenaemia reported a clinical history of thrombosis. A number of additional PLG polymorphisms and clinically significant dysplasminogenaemias have also been reported (Robbins, 1990).

Mutations in tPA or urokinase have not been clinically linked to thrombophilia. However, defects in PLG activator release from the vessel wall, as well as increased inhibition of tPA by PAI-1, have both been associated with a thrombotic diathesis. Increased circulating PAI-1 appears to represent an independent risk factor for vascular re-occlusion in young survivors of myocardial infarction (Hamsten *et al*, 1985). In addition, increased levels of PAI-1 have been associated with deep vein thrombosis in patients undergoing hip replacement surgery and in individuals with insulin resistance. However, PAI-1 is itself an acute phase reactant, and thus may not be directly responsible for the observed prothrombotic tendency (Hajjar, 2003a).

In a large prospectively studied cohort of individuals, high plasma levels (≥75th percentile) of the fibrinolytic attenuator, TAFI, have been associated with a twofold higher risk for recurrence of an initial venous thromboembolism. Patients with high TAFI levels had significantly higher levels of coagulation factors XI, VIII and IX, and a higher risk of recurrence was observed among patients with high levels of both TAFI and one of these factors (Eichinger *et al*, 2004). The association of arterial thrombosis with elevated TAFI levels or that of particular TAFI polymorphisms with venous thrombosis has not yet been clearly established.

Recently, the Leiden Thrombophilia Study (LETS) showed that plasma hypofibrinolysis is a risk factor for venous thrombosis. In their study, a twofold increase risk of deep venous thrombosis was seen in individuals with clot lysis times above the 90th percentile of levels present in the healthy population (Listman *et al*, 2005).

### *Enhanced fibrinolysis and bleeding*

Enhanced fibrinolysis because of congenital or acquired loss of fibrinolytic inhibitor activity is associated with a bleeding diathesis. When clotted *in vitro* in the presence of tPA, plasmas deficient in intrinsic pathway factors VIII (haemophilia A), IX (haemophilia B), lyse substantially faster than normal plasma. Premature lysis may reflect insufficient thrombin generation during the clotting process, leading to suboptimal activation of TAFI, and early thrombus dissolution. Therefore, bleeding in patients with haemophilia may be due to both a failure of clot formation and a failure of TAFI activation, with subsequent excessive fibrinolysis (Nesheim, 2003). Patients with congenital deficiency of $\alpha_2$-PI also have premature lysis of the haemostatic plug (Saito, 1988). Acquired $\alpha_2$-PI deficiency may be seen in patients with severe liver disease because of decreased synthesis, disseminated intravascular coagulation as result of consumption, nephrotic syndrome as a result of urinary losses, or during thrombolytic therapy, which induces excessive utilization of the inhibitor.

Patients with acute promyelocytic leukaemia frequently develop severe bleeding accompanied by accelerated plasmin generation and $\alpha_2$-antiplasmin depletion. In this disorder, leukaemic blasts overexpress annexin 2 and support enhanced cell surface plasmin generation (Menell *et al*, 1999). Bleeding resolves upon initiation of all-*trans*-retinoic acid therapy, which eliminates expression of annexin 2 through a transcriptional mechanism.

In a child with complete loss of PAI-1 expression, an autosomal recessive frame-shift mutation induced a premature stop codon in exon 4. This patient exhibited severe haemorrhage, but only in the setting of trauma or surgery (Fay *et al*, 1992). These findings suggest that the function of PAI-1 in humans may be limited to regulation of fibrinolysis.

## Conclusions

Fibrinolysis is a highly regulated system that integrates with the coagulation system through several direct molecular links. The major proteins involved in PLG activation have been identified and characterized at the molecular level. Mice deficient in many of these molecules have been developed, providing insight into their functional roles *in vivo*. Although the specific mechanisms leading to plasmin generation are common to both intravascular and extravascular settings, the physiological and pathophysiological effects of plasmin generation are context-dependent and sometimes unexpected. Challenge of genetically engineered mice has illustrated the broad activities of this system relating to haemostasis, tumour progression, inflammation, vascular remodelling, angiogenesis and host defence. While such studies will illuminate our understanding of human physiology, we must bear in mind the differences between murine and human fibrinolysis. While primary genetic disorders of fibrinolysis are uncommon, they have

been clearly linked to bleeding and thrombosis. Acquired fibrinolytic disorders, on the other hand, are frequent, and secondary to other primary disorders or therapeutic interventions.

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