[Frontiers in Bioscience 8, d1-8, January 1, 2003]

REGULATION OF PLASMINOGEN RECEPTORS

Thomas Herren, [1] Carmen Swaisgood, [2] Edward F. Plow [3]

[1]Dept of Medicine, Spital Limmattal, Schlieren, ZH, Switzerland, [2,3]Joseph J. Jacobs Center for Thrombosis and Vascular Biology, Department of Molecular Cardiology, Cleveland Clinic Foundation, Cleveland, OH

TABLE OF CONTENTS

1. ABSTRACT

Many eukaryotic and prokaryotic cells bind plasminogen in a specific and saturable manner. When plasminogen is bound to cell-surface proteins with C-terminal lysines via its lysine binding sites, its activation to plasmin is accelerated, and cell-bound plasmin is protected from inactivation by natural inhibitors. Plasmin mediates direct or indirect degradation of the extracellular matrix, and bound plasmin is used by cells to facilitate migration through extracellular matrices. Since cell migration and tissue remodelling are the underpinnings of many physiological and pathological responses, the modulation of plasminogen receptors may serve as a primary regulatory mechanism for control of many cellular responses. Specific examples of cell types on which plasminogen receptors undergo modulation include: fibroblasts, where modulation may contribute to cartilage and bone destruction in rheumatoid arthritis; leukemic cells, where enhanced plasminogen binding may contribute to the heightened fibrinolytic state in the patients; other tumor cells, where up-regulation may support invasion and metastasis; bacteria, where enhanced plasminogen binding may facilitate tissue destruction and invasion; platelets, where up-regulation of plasminogen binding may play a role in regulating clot lysis; and adipocytes, where the modulation of plasminogen receptor expression may regulate cell differentiation and fat accumulation. Two pathways for modulation of plasminogen receptors have been characterized: A protease-dependent pathway can either up-regulate or down-regulate plasminogen binding to cells by changing the availability of plasminogen-binding proteins with C-terminal lysines. New receptors may be generated by trypsin-like proteases, including plasmin, which create new C-terminal lysines; other enzymes may expose existing membrane proteins by altering the cell surface; or receptor function may be lost by removal of C-terminal lysines. The basic carboxypeptidases of blood carboxypeptidase N and plasma carboxypeptidase B (TAFI) mediate such down-regulation. A non-protease dependent pathway for modulation of plasminogen receptors may be initiated by growth factors, chemokines or cytokines that alter the cell membrane and/or cytoskeleton architectures to expose plasminogen binding sites. Many examples of the modulation of plasminogen receptors have been demonstrated *in vitro*, and the development of knock-out mice may soon lead to incisive evaluations of the significance of the regulation of plasminogen receptors *in vivo.*

2. INTRODUCTION

Plasmin is a protease with broad substrate recognition. In addition to fibrin, plasmin cleaves many

extracellular matrix proteins, including fibronectin (1), von Willebrand factor (2), laminin (3)and thrombospondin (4) and can activate many of the matrix metalloproteinases (MMP) (5,6), which degrade still other matrix constituents. Plasmin also can affect the activity of cytokines and growth factors, notably TGF-beta (7,8), which influences the composition of the extracellular matrix. Therefore, to maintain tissue homeostasis and avoid frank tissue damage, plasmin activity must be tightly controlled. Such regulation is achieved at multiple checkpoints within the plasminogen system. The availability of the plasminogen activators and the inhibitors of these activators plays a primary role in regulating plasmin formation. Direct suppression of plasmin activity is determined primarily by $alpha_2$-antiplasmin (9,10); and additional protease inhibitors (11) provide an emergency backup network.

Plasminogen and plasmin bind to receptors on a variety of cells, including leukocytes, endothelial cells, platelets, fibroblasts, epidermal cells, neuronal cells and hepatocytes (reviewed in (12-14)). Binding of plasminogen to its cellular receptors is mediated by its lysine-binding sites in its heavy chain region, specifically those associated with the kringle 1 and 5 domains of plasminogen (15). These structures recognize cell surface constituents with c-terminal lysines or mimics of this motif (16,17). The binding of plasminogen to cell surfaces controls the rate of plasminogen activation and susceptibility of plasmin to inactivation by inhibitors (18-20). Hence, by interacting with cells, plasminogen activation and plasmin activity can be spatially and temporally directed according to cellular needs. Consequently, the plasminogen system has been implicated in mediating cell migration in processes, such as atherosclerosis, restenosis and inflammation (reviewed in (21,22)). With the broad biological significance of the interaction of plasminogen and plasmin with cellular receptors, the regulation of these receptors may be of paramount importance in controlling the function of the plasminogen system. Changes in receptor number or affinity could exert profound effects on controlling cellular responses influenced by plasmin(ogen). The regulation of plasminogen receptors is the focus of this review.

3. EXAMPLES OF REGULATION OF PLASMINOGEN RECEPTORS

3.1. Rheumatoid arthritis

The plasminogen system has been shown to be of major importance in inflammatory joint diseases, including rheumatoid arthritis (23). The pannus, which is pathognomonic for this disease, consists of proliferating fibroblasts and inflammatory cells and invades cartilage and bone. Cartilage damage is primarily mediated by fibroblasts and macrophages that harness MMP and other proteases, including plasmin. Plasmin may directly degrade cartilage proteoglycans and can activate MMPs with collagenase activities and neutral proteoglycanases (24). Interestingly, synovial fibroblasts from patients with rheumatoid arthritis have substantially higher binding capacities for plasminogen compared to normal synovial fibroblasts ($59x10^6$ vs. $29x10^6$ plasminogen molecules bound/cell). It has been postulated that this up-regulation of the plasminogen binding capacity of fibroblasts, which in turn leads to greater plasmin formation, may be key to the destruction of joint cartilage and bone observed in patients with rheumatoid arthritis. Gonzalez-Gronow *et al.* (25) reported that enhanced plasminogen binding to rheumatoid arthritis fibroblasts is mediated by a glycoprotein related to a platelet membrane protein, integrin alpha IIb beta III (GPIIb-IIIa), in association with a 130-kDa protein, which is antigenetically related to the alpha2-macroglobulin receptor-associated protein, and dipeptidyl peptidase IV.

3.2. Leukemia

An important feature of acute promyelocytic leukemia (APL) is a hemorrhagic diathesis, which may be due to increased activity of the plasminogen system. Low plasma concentrations of plasminogen and $alpha_2$-antiplasmin have been reported in APL patients, which may reflect consumption of these components. The hemorrhagic diathesis is clinically relevant, especially in patients having the chromosomal translocation t(15;17). Interestingly, plasminogen activation was significantly enhanced on APL cells with this translocation (NB4 cells) compared to APL cells without the translocation or other forms of leukemic cells (AML-M1). The increase in plasminogen activation could be blunted by addition of the carboxy-terminal lysine analogue, epsilon-aminocaproic acid (EACA), suggesting that binding of plasminogen to the cell surface is involved in its activation. In addition, an antibody to annexin II, a known plasminogen receptor (26), also diminished plasminogen activation, which is consistent with a role of this receptor in plasminogen binding to leukemic cells. The presence of annexin II on the surface of APL cells was confirmed by immunofluorescence studies. Taken together, these results suggest that increased expression of plasminogen receptors may lead to increased plasmin production, which may even become systemically active and contribute to the increased risk of bleeding in leukemic patients (27). In general, if one compares the binding capacity of normal white cell populations, neutrophils, monocytes and lymphocytes, to transformed cells lines corresponding to these cell types, the plasminogen binding capacity of the transformed cells is 2-3 orders of magnitude greater (12,18).

3.3. Tumor Invasion and Metastasis

Burtin and colleagues (28,29) emphasized that tumor cells originating from a human colonic carcinoma have high plasmin binding capacities and that plasmin could enhance its own binding to tumor cells. Cancer cells may use plasmin bound to their surface to degrade extracellular matrices, thereby facilitating their invasion of tissue and metastasis to distant sites. Ranson *et al.* (30) have shown that a metastatic breast cancer cell line (MDA-MB-231) has a higher plasminogen binding capacity compared to non-metastatic cancer cells (MCF-7 and T-47D) and that the metastatic cells converted plasminogen much more efficiently to plasmin. Therefore, increased expression of plasminogen receptors on breast cancer cells may foster their potential to metastasize. One important plasminogen receptor on breast cancer cells was shown to be cytokeratin 8 (31). This receptor is also expressed on other malignant cells of both epithelial (squamous cell

carcinoma) and non-epithelial (melanoma, lymphoma, glioma, sarcoma) origin. It is not known whether blockade of this receptor, e.g. by antibodies, will reduce metastatic potential. However, human urinary trypsin inhibitor was shown to inactivate receptor-bound plasmin in ovarian cancer cells, choriocarcinoma cells and a lung cancer cell line; and this inactivation of bound plasmin inhibited cell invasion in an *in vitro* assay and reduced the number of lung metastases *in vivo* (32). Also, EACA has been shown to reduce invasion of colon cancer cells, a result indicative of the importance of plasminogen binding in mediating cellular infiltration (33). These results suggest that modulation of the expression of plasminogen receptors has distinct clinical effects.

Many chemotherapeutic regimens include glucocorticoids. Pöllänen (34) showed that fibrosarcoma cells significantly decreased plasminogen binding after glucocorticoid treatment. The decrease was apparently due to a decrease in affinity for plasminogen (5nM dexamethasone; K_d decreased from $5.4x10^{-9}$M to $1.2x10^{-7}$M). By this mechanism, cells are disarmed of a major constituent of their proteolytic activity, and thereby invade tissue barriers less effectively. This alteration may slow tumor growth and/or metastasis. This latter example provides one of the rare instances in which modulation of plasminogen binding arises as a consequence of a change in receptor affinity. In most examples, including those cited above, it is the number of plasminogen receptors that undergoes modulation.

3.4. Bacterial Disease

Many bacteria express plasminogen receptors (reviewed in (35,36)). For example, Salmonella or Borrelia bind plasminogen to their surface, where it is activated to plasmin and potentiates bacterial penetration through basement membranes or endothelial monolayers. Addition of aprotinin significantly impairs bacterial penetration, indicating that the microorganisms may use plasmin to invade tissue barriers (37,38). This concept has been recently corroborated in a mouse skin infection model using group A streptococci: acquisition of plasmin activity is associated with increased virulence of the bacteria (39). In an animal peritonitis model, intraperitoneal administration of aprotinin improves survival and reduces the number of abscesses formed (40). Several bacterial plasminogen receptors have been identified (35,36,41). One of these is lactic acid dehydrogenase (42), suggesting a novel regulatory mechanism for this glycolytic enzyme with a c-terminal lysine to attain surface expression.

3.5. Thrombolysis

The two major constituents of a thrombus are fibrin and platelets. Plasminogen is capable of binding to both of these components. Resting platelets are capable of binding approximately 40,000 plasminogen molecules per cell with the integrin alpha IIb beta III (GPIIb-IIIa) serving as the receptor. When platelets are stimulated with thrombin, a major platelet agonist, they increase their plasminogen binding capacity by approximately 5-fold (43,44). Using platelets from patients with congenital deficiencies, it was shown that fibrinogen released from within platelets and expressed on the platelet surface as fibrin accounts for the increase in plasminogen binding sites (44). Other constituents of platelet alpha-granules, including thrombospondin-1 (45) and fibronectin (1), have the capability of binding to plasminogen and to the platelet surface but do not appear to substantially enhance surface expression of plasminogen on stimulated platelets.

3.6. Adipose Tissue Development

Plasminogen deficient mice have reduced body weight compared to age and sex-matched wild-type animals (46,47). This difference is not only observed at later stages of life when the plasminogen-deficient mice develop a wasting syndrome (47) but is observed during the growth phase in the animals and is due to a reduced rate of adipose tissue and whole body fat accumulation (46). These differences can be traced to differences in the stromal cell populations in plasminogen-deficient and wild-type animals and their capacity to differentiate. In examining the plasminogen binding capacity of a model of differentiated and undifferentiated adipocytes, dexamethasone and insulin stimulated and nonstimulated 3T3 cells, the number of plasminogen binding sites increased 10-fold on the differentiated cells. Consistent with this finding, plasminogen binding was higher to mature fat cells and differentiated stromal cells than to undifferentiated stromal cells isolated from adipose tissues in mice (48). Thus, plasminogen receptor expression appears to increase with adipocyte differentiation. A concept emerging from studies of mice deficient in various components of the plasminogen system is that this pathway plays a major regulatory role in adipose tissue development (49).

4. PATHWAYS OF PLASMINOGEN RECEPTOR MODULATION

4.1. Protease-dependent Pathways

Enzymatic remodeling provides a rapid mechanism for cells to alter their surface properties. By this mechanism, new receptors may become activated or accessible while others are inactivated by membrane-bound enzymes. When neutrophils or monocytes are isolated and cultured overnight, they up-regulate dramatically (~30-fold) their expression of plasminogen receptors (50,51). The culturing may be a model for migration of these phagocytic cells from the blood to extravascular sites of inflammation. In exploring this phenomenon with neutrophils, the up-regulation was prevented by addition of protease inhibitors. Using inhibitors of different specificity, elastase and/or the closely related enzyme, proteinase 3, and cathepsin G were implicated in the up-regulation of plasminogen binding of the cultured neutrophils. The increase in plasminogen binding to the cultured neutrophils is due to an increase in the number of receptors, and the newly exposed receptors bind to the lysine binding sites of plasminogen. Nevertheless, all three of the implicated enzymes do not cleave substrates to create new c-terminal lysines. Therefore, these proteases appear to enhance plasminogen binding by remodeling the cell surface to enhance the accessibility to plasminogen binding sites. This proposition is modeled in Figure 1A. In support of this interpretation, protease treatment of neutrophils with elastase or cathepsin

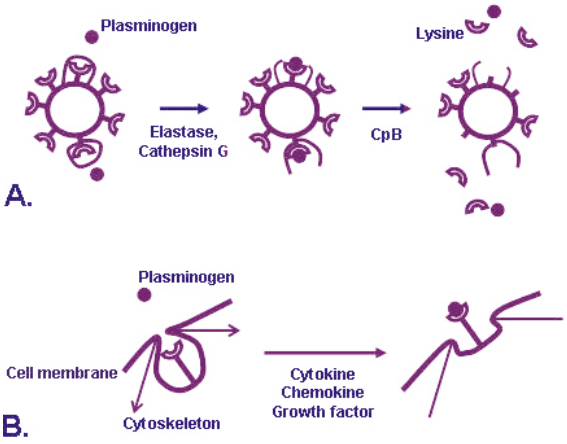

Figure 1. Pathways for modulation of Plasminogen Receptor Expression. (A) Protease-dependent Pathways. By cleaving substrates on the cell surface, proteases, such as elastase, cathepsin G and proteinase 3, can expose plasminogen binding sites and, thereby, increase the plasminogen binding capacity of cells. Plasmin can directly generate new carboxy-terminal lysines to enhance plasminogen binding to cells. Removal of carboxy-terminal lysines by the basic carboxypeptidases renders the receptors incompetent to bind plasminogen, and the cellular binding capacity decreases. (B) Protease-independent Pathways. Cells frequently respond to stimuli, such as chemokines, cytokines and growth factors, by altering their morphology. Such changes in shape are often a consequence of the assembly and spatial reorganization of the actin cytoskeleton. At the cell-surface, such changes may alter the availability of plasminogen receptors independent of proteolytic events. The up-regulation of plasminogen binding induced by cell adhesion or by PMA-stimulation of neutrophils may be a consequence of protease-independent pathways.

G significantly increased their plasminogen binding capacity (52). It is well-established that neutrophils express both elastase and cathepsin G on their surface and that exposure of the cells to chemoattractants, such as fMLP or C5a, or to TNF-alpha or platelet-activating factor, increases this expression (53,54). The source of this increased expression is the release of the enzymes from their stores within the azurophilic granules of the cells (55). Thus, there is a ready reservoir of enzymatic activity available to remodel the membrane surface and enhance plasminogen binding to these cells.

Trypsin-like enzymes cleave protein substrates on cell surfaces to generate new carboxy-terminal lysines, which can serve directly as new plasminogen binding sites (Figure 1A). Plasmin itself falls into this category, creating a positive feedback loop for regulating its own binding to cells. Gonzalez-Gronow *et al.* (56) reported that plasmin induced a 3-fold increase in plasminogen binding to U937 cells ($1.1x10^9$ sites/cell vs. $4.3x10^8$) (56). Camacho *et al.*, (29) showed that plasmin or trypsin treatment of colonic carcinoma cells increased their plasmin binding by 2-5 fold. It is becoming increasingly evident that tumors produce a variety of proteases. One of the possible functions of these proteases is to remodel the tumor cell surface to enhance plasminogen binding and activation, which would facilitate tissue invasion, metastasis and angiogenesis.

While each of the enzymatic processes described above results in an up-regulation of plasminogen binding to cells, enzymatic regulation can also dampen plasminogen receptor expression. The basic carboxypeptidases, which remove carboxy-terminal lysines from substrates, function in such a suppressive role. The two major basic carboxypeptidases of blood are CpN and CpB (also identified as CpU, CpR and TAFI). CpN is constitutively active (57), whereas CpB circulates as a proenzyme that must be enzymatically activated to acquire carboxypeptidase activity (58,59). Plasmin and thrombin can activate the zymogen form of CpB but the physiological activator is likely to be the thrombin-thrombomodulin complex (60). Both CpN and CpB remove carboxy-terminal lysines from cell surfaces and, thereby, decrease the plasminogen binding capacity of cells (Figure 1A). Treatment of cells with either carboxypeptidase decreases the plasminogen binding capacity of cells (61). A suppressive effect has been observed with monocytoid, lymphoblastoid cells and neutrophils. Although thrombin enhances plasminogen binding to platelets (43), it reduces the plasminogen binding capacity of endothelial cells (62).

4.2. Protease-independent Pathways

Stimulation of neutrophils with PMA leads to a concentration-dependent, rapid increase in their plasminogen binding capacity. Although PMA up-regulates elastase and cathepsin G on the surface of neutrophils (54), this increase in plasminogen binding was insensitive to the protease inhibitors, which block the culture-induced up-regulation in plasminogen binding capacity described above. Therefore, either PMA induces other proteases, or a distinct mechanism is operative. The rapidity of the up-regulation favors a non-enzymatic mechanism, such as a change in the architecture of the plasma membrane and/or cytoskeleton to create new and/or more plasminogen binding sites on the cell surface (Figure 1B).

PMA treatment of U937 and THP-1 monocytoid cells also alters their plasminogen binding capacities. When these cells are stimulated with PMA and are provided with a limited surface, an adherent and non-adherent subpopulation can be separated (63). Plasminogen receptors are up-regulated in the non-adherent cells by up to 17-fold compared to non-stimulated control cells. The presence of an adherent cell population is required for the up-regulation to occur in the nonadherent cells. In normal peripheral human monocytes, however, PMA stimulation results in an up-regulation of plasminogen receptors without requirement for an adherent population. When monocytoid cells are exposed to extracellular matrix proteins, such as fibronectin, vitronectin, or laminin, an up-regulation in plasminogen binding capacity can again be measured in the non-adherent cell population. Compared to control cells, the binding capacity is decreased in the

adherent cells (64). Functionally, the increased plasminogen binding capacity enables the non-adherent cells to promote plasminogen activation at an enhanced rate. The up-regulation of plasminogen receptors was susceptible to carboxypeptidase treatment, indicating that the newly exposed carboxy-terminal lysines mediate the binding of plasminogen. Still other agonists have been shown to enhance plasminogen binding to monocytoid cells (65). Among these are 1,25-dihydroxyvitamin D and interferon-gamma, which induce differentiation of these cells.

The membrane-type MMP1, another enzyme capable of digesting extracellular matrix proteins, was reported to be modulated by clathrin-mediated endocytosis (66,67). Modulation of the cell membrane, therefore, is a versatile and well-established mechanism to regulate enzymatic activity. Alcohol modulates the lipid bilayer of the cell membrane, and moderate alcohol consumption is statistically associated with a lower cardiovascular morbidity and mortality. Treatment of human umbilical vein endothelial cells with ethanol, 0.1% (v/v), increased their plasminogen binding capacity by 3-fold and their ability to generate plasmin. The increase was associated with increased expression of annexin II on the surface of the endothelial cells (68). Therefore, the increased fibrinolytic potential on endothelial cells may translate into a clinically beneficial effect.

5. PERSPECTIVE: MODULATION OF PLASMINOGEN RECEPTORS *IN VIVO*

The next frontier in our understanding of the role of plasminogen receptors and their modulation is likely to be their manipulation in mice. Currently, *in vivo* regulation of plasminogen receptors in such over-expression or knock-out models has yet to be described in detail. However, the groundwork for such studies has been established from studies conducted in plasminogen-deficient mice (69,70), which have demonstrated a clear role of plasminogen in cell migration. In particular, the recruitment of leukocytes is diminished in plasminogen-deficient compared to wild-type animals. In the thioglycollate peritonitis model, monocyte/macrophage and lymphocyte recruitment was blunted in the plasminogen-deficient mice, indicating a role for plasminogen in the inflammatory response (71). In mouse models of transplant atherosclerosis (72) and restenosis (73), early infiltration of monocyte/macrophages into the vessel wall also was blunted in the plasminogen-deficient mice compared to wild-type animals. This decrease in leukocyte migration may have been the reason for reduced neointimal development in the plasminogen-deficient mice in these models (73-75). In the mouse restenosis model, migration of smooth muscle cells was also significantly reduced in plasminogen deficient mice (73). Some suggestive evidence that plasminogen receptors per se may be involved in inflammatory cell migration has also been developed. Plasminogen binding was measured by FACS to neutrophils recruited into the peritoneal cavity in response to thioglycollate compared to neutrophils recovered from blood. The amount of plasminogen bound to the migrated cells was 3-fold greater (51). A preliminary report suggests that inactivation of the gene for annexin II in mice leads to an impairment of fibrinolysis (76). Finally, in a human study, cell-bound plasminogen could be detected on macrophages recovered from patients undergoing peritoneal dialysis (77). These data also point to a role of plasminogen receptors in inflammatory disorders in humans.

6. ACKNOWLEDGMENTS

This work was supported by NIH grant HL-17964. to EFP. CS is supported by NIH Training Grant T32 HL07914. The authors express thanks to Jane Rein for expert secretarial assistance.

7. REFERENCES

1.Salonen, E. V., O. Sakeseki, T. Vartio, A. Vaheri, L. S. Neilsen, & J. Seuthen: Plasminogen and tissue-type plasminogen activator bind to immobilized fibronectin. *J Biol Chem* 260, 12302-12307 (1985)

1.Bonnefoy, A. & C. Legrand: Proteolysis of subendothelial adhesive glycoproteins (fibronectin, thrombospondin, and von Willebrand factor) by plasmin, leukocyte cathepsin G, and elastase. *Thromb Res* 98, 323-332 (2000)

1.Liotta, L. A., R. H. Goldfarb, G. P. Brundage, G. P. Siegal, V. Terranova, & S. Garbisa: Effect of plasminogen activator (urokinase), plasmin, and thrombin on glycoprotein and collagenous components of basement membrane. *Cancer Res* 41, 4629-4636 (1981)

4. Silverstein, R. L. & R. L. Nachman: Thrombospondin-plasminogen interactions: Modulation of plasmin generation. *Semin Thromb Hemost* 13, 335-342 (1987)

5. Lijnen, H. R.: Molecular interactions between the plasminogen/plasmin and matrix metalloproteinase systems. *Fibrinolysis & Proteolysis* 14, 175-181 (2000)

6. Lijnen, H. R.: Plasmin and matrix metalloproteinases in vascular remodeling. *Thromb Haemost* 86, 324-333 (2001)

7. Saksela, O. & D. B. Rifkin: Cell-associated plasminogen activation: Regulation and physiological functions. *Annu Rev Cell Biol* 4, 93-126 (1988)

8. Lyons, R. M., L. E. Gentry, A. F. Purchio, & H. L. Moses: Mechanism of activation of latent recombinant transforming growth factor beta 1 by plasmin. *J Cell Biol* 110, 1361-1367 (1990)

9. Collen, D.: Identification and some properties of a new fast-reacting plasmin inhibitor in human plasma. *Eur J Biochem* 69, 209-216 (1976)

10. Aoki, N., M. Moroi, M. Matsuda, & K. Tachiya: The behavior of alpha$_2$-plasmin inhibitor in fibrinolytic states. *J Clin Invest* 60, 361-369 (1977)

11. Aoki, N. & P. C. Harpel: Inhibitors of the fibrinolytic enzyme system. *Semin Thromb Hemost* 10, 24-41 (1984)

12. Miles, L. A. & E. F. Plow: Receptor mediated binding of the fibrinolytic components, plasminogen and urokinase, to peripheral blood cells. *Thromb Haemost* 58, 936-942 (1987)

13. Hajjar, K. A.: Cellular receptors in the regulation of plasmin generation. *Thromb Haemost* 74, 294-301 (1995)

14. Plow, E. F., A. Redlitz, S. B. Hawley, S. Xue, T. Herren, J. L. Hoover-Plow, & L. A. Miles: Assembly of the

plasminogen system on cell surfaces. In: F. Bachmann (ed.), Handbook of Experimental Pharmacology, Volume 146, 141-170, Springer, Berlin (2000)
15. Miles, L. A., C. M. Dahlberg, & E. F. Plow: The cell-binding domains of plasminogen and their function in plasma. *J Biol Chem* 263, 11928-11934 (1988)
16. Miles, L. A., C. M. Dahlberg, J. Plescia, J. Felez, K. Kato, & E. F. Plow: Role of cell-surface lysines in plasminogen binding to cells: identification of alpha-enolase as a candidate plasminogen receptor. *Biochemistry* 30, 1682-1691 (1991)
17. Miles, L. A., C. M. Dahlberg, E. G. Levin, & E. F. Plow: Gangliosides interact directly with plasminogen and urokinase and may mediate binding of these fibrinolytic components to cells. *Biochemistry* 28, 9337-9343 (1989)
18. Plow, E. F., D. E. Freaney, J. Plescia, & L. A. Miles: The plasminogen system and cell surfaces: Evidence for plasminogen and urokinase receptors on the same cell type. *J Cell Biol* 103, 2411-2420 (1986)
19. Hall, S. W., J. E. Humphries, & S. L. Gonias: Inhibition of cell surface receptor-bound plasmin by $alpha_2$-antiplasmin and $alpha_2$-macroglobulin . *J Biol Chem* 266, 12329-12336 (1991)
20. Gong, Y., S. O. Kim, J. Felez, D. K. Grella, F. J. Castellino, & L. A. Miles: Conversion of glu-plasminogen to lys-plasminogen is necessary for optimal stimulation of plasminogen activation on the endothelial cell surface. *J Biol Chem* 276, 19078-19083 (2001)
21. Plow, E. F., V. A. Ploplis, S. Busuttil, P. Carmeliet, & D. Collen: A role of plasminogen in atherosclerosis and restenosis models in mice. *Thromb Haemost* 82 (Suppl. 1), 4-7 (1999)
22. Plow, E. F., V. A. Ploplis, P. Carmeliet, & D. Collen: Plasminogen and cell migration *in vivo*. *Fibrinolysis & Proteolysis* 13, 49-53 (1999)
23. Del Rosso, M., G. Fibbi, & M. Matucci Cerinic: The urokinase-type plasminogen activator system and inflammatory joint diseases. *Clin Exp Rheumatol* 17, 485-498 (1999)
24. Gonzalez-Gronow, M., G. Gawdi, & S. V. Pizzo: Plasminogen activation stimulates an increase in intracellular calcium in human synovial fibroblasts. *J Biol Chem* 268, 20791-20795 (1993)
25. Gonzalez-Gronow, M., G. Gawdi, & S. V. Pizzo: Characterization of the plasminogen receptors of normal and rheumatoid arthritis human synovial fibroblasts. *J Biol Chem* 269, 4360-4366 (1994)
26. Cesarman, G. M., C. A. Guevara, & K. A. Hajjar: An endothelial cell receptor for plasminogen/tissue plasminogen activator (t-PA). II. Annexin II-mediated enhancement of t-PA-dependent plasminogen activation. *J Biol Chem* 269, 21198-21203 (1994)
27. Menell, J. S., G. M. Cesarman, A. T. Jacovina, M. A. McLaughlin, E. A. Lev, & K. A. Hajjar: Annexin II and bleeding in acute promyelocytic leukemia. *N Engl J Med* 340, 994-1004 (1999)
28. Burtin, P., G. Chavanel, J. Andre-Bougaran, & A. Gentile: The plasmin system in human adenocarcinomas and their metastases. A comparative immunofluorescence study. *Int J Cancer* 39, 170-178 (1987)
29. Camacho, M., M.-C. Fondanèche, & P. Burtin: Limited proteolysis of tumor cells increases their plasmin-binding ability. *FEBS Lett* 245, 21-24 (1989)
30. Ranson, M., N. M. Andronicos, M. J. O'Mullane, & M. S. Baker: Increased plasminogen binding is associated with metastatic breast cancer cells: differential expression of plasminogen binding proteins. *Br J Cancer* 77, 1586-1597 (1998)
31. Hembrough, T. A., L. Li, & S. L. Gonias: Cell-surface cytokeratin 8 is the major plasminogen receptor on breast cancer cells and is required for the accelerated activation of cell-associated plasminogen by tissue-type plasminogen activator. *J Biol Chem* 271, 25684-25691 (1996)
32. Kobayashi, H., H. Shinohara, K. Takeuchi, M. Itoh , M. Fujie, M. Saitoh, & T. Terao: Inhibition of the soluble and the tumor cell receptor-bound plasmin by urinary trypsin inhibitor and subsequent effects on tumor cell invasion and metastasis. *Cancer Res* 54, 844-849 (1994)
33. Schlechte, W., G. Murano, & D. Boyd: Examination of the role of the urokinase receptor in human colon cancer mediated laminin degradation. *Cancer Res* 49, 6064-6069 (1989)
34. Pollanen, J.: Down-regulation of plasmin receptors on human sarcoma cells by glucocorticoids. *J Biol Chem* 264, 5628-5632 (1989)
35. Boyle, M. D. P. & R. Lottenberg: Plasminogen activation by invasive human pathogens. *Thromb Haemost* 77, 1-10 (1997)
36. Ullberg, M., G. Kronvall, I. Karlsson, & B. Wiman : Receptors for human plasminogen on gram-negative bacteria. *Infect Immun* 58, 21-25 (1990)
37. Lahteenmaki, K., R. Virkola, R. Pouttu, P. Kuusela, M. Kukkonen, & T. K. Korhonen : Bacterial plasminogen receptors: *in vitro* evidence for a role in degradation of the mammalian extracellular matrix. *Infect Immun* 63, 3659-3664 (1995)
38. Coleman, J. L., T. J. Sellati, J. E. Testa, R. R. Kew, M. B. Furie, & J. L. Benach: *Borrelia burgdorferi* binds plasminogen, resulting in enhanced penetration of endothelial monolayers. *Infect Immun* 63, 2478-2484 (1995)
39. Li, Z., V. A. Ploplis, E. L. French, & M. D. Boyle: Interaction between group A streptococci and the plasmin(ogen) system promotes virulence in a mouse skin infection model. *J Infect Dis* 179, 907-914 (1999)
40. Chalkiadakis, G. E., A. Kostakis, P. Karydakis, M. E. Chalkiadakis, P. Matsikas, P. E. Karayannoccos, M. Sechas, & G. D. Skalkeas: Effect of aprotinin on fibrinopurulent peritonitis in rats. *Am J Surg* 150, 550-553 (1985)
41. Berge, A. & U. Sjöbring: PAM, a novel plasminogen-binding protein from *Streptococcus pyogenes*. *J Biol Chem* 268, 25417-25424 (1993)
42. Lottenberg, R., C. C. Broder, M. D. P. Boyle, S. J. Kain, B. L. Schroeder, & I. Curtiss: Cloning, sequence analysis, and expression in escherichia coli of a streptococcal plasmin receptor. *J Bacteriol* 174, 5204-5210 (1992)
43. Miles, L. A. & E. F. Plow: Binding and activation of plasminogen on the platelet surface. *J Biol Chem* 260, 4303-4311 (1985)
44. Miles, L. A., M. H. Ginsberg, J. G. White, & E. F. Plow: Plasminogen interacts with human platelets through two distinct mechanisms. *J Clin Invest* 77, 2001-2009 (1986)

45. Silverstein, R. L., L. L. K. Leung, P. C. Harpel, & R. L. Nachman: Complex formation of platelet thrombospondin with plasminogen. *J Clin Invest* 74, 1625-1633 (1984)
46. Hoover-Plow, J., J. Ellis, & L. Yuen: *In vivo* plasminogen deficiency reduces fat accumulation. *Thromb Haemost* 87, 1011-1019 (2002)
47. Bugge, T. H., K. W. Kombrinck, M. J. Flick, C. C. Daugherty, M. J. S. Danton, & J. L. Degen: Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. *Cell* 87, 709-719 (1996)
48. Hoover-Plow, J. & L. Yuen: Plasminogen binding is increased with adipocyte differentiation. *Biochem Biophys Res Commun* 284, 389-394 (2001)
49. Samad, F. & D. J. Loskutoff: Hemostatic gene expression and vascular disease in obesity: insights from studies of genetically obese mice. *Thromb Haemost* 82, 742-747 (1999)
50. Felez, J., L. A. Miles, P. Fabregas, M. Jardi, E. F. Plow, & R. H. Lijnen: Characterization of cellular binding sites and interactive regions within reactants required for enhancement of plasminogen activation by t-PA on the surface of leukocytic cells. *Thromb Haemost* 76, 577-584 (1996)
51. Herren, T., T. A. Burke, M. Jardi, J. Felez & E. F. Plow: Regulation of plasminogen binding to neutrophils. *Blood* 97, 1070-1078 (2001)
52. Herren, T., T. A. Burke, & E. F. Plow: Mechanisms for modulation of plasminogen binding to cell surfaces: role of a newly identified plasminogen receptor, histone protein H2B. *J Biol Chem* (under revision)
53. Owen, C. A., M. A. Campbell, S. S. Boukedes, & E. J. Campbell: Inducible binding of bioactive cathepsin G to the cell surface of neutrophils. A novel mechanism for mediating extracellular catalytic activity of cathepsin G. *J Immunol* 155, 5803-5810 (1995)
54. Owen, C. A., M. A. Campbell, P. L. Sannes, S. S. Boukedes, & E. J. Campbell: Cell surface-bound elastase and cathepsin G on human neutrophils: a novel, non-oxidative mechanism by which neutrophils focus and preserve catalytic activity of serine proteases. *J Cell Biol* 131, 775-789 (1995)
55. Borregaard, N. & J. B. Cowland: Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 89, 3503-3521 (1997)
56. Gonzalez-Gronow, M., S. Stack, & S. V. Pizzo: Plasmin binding to the plasminogen receptor enhances catalytic efficiency and activates the receptor for subsequent ligand binding. *Arch Biochem Biophys* 286, 625- 628 (1991)
57. Skidgel, R. A.: Human carboxypeptidase N (lysine carboxypeptidase). *Methods Enzymol* 248, 653-663 (1995)
58. Eaton, D. L., B. E. Malloy, S. P. Tsai, W. Henzel , & D. Drayna: Isolation, molecular cloning, and partial characterization of a novel carboxypeptidase B from human plasma. *J Biol Chem* 266, 21833-21838 (1991)
59. Nesheim, M. E.: TAFI. *Fibrinolysis & Proteolysis* 13, 72-77 (1999)
60. Bajzar, L., M. E. Nesheim, & P. B. Tracy: The profibrinolytic effect of activated protein C in clots formed from plasma is TAFI-dependent. *Blood* 88, 2093-2100 (1996)
61. Redlitz, A., A. K. Tan, D. L. Eaton, & E. F. Plow : Plasma carboxypeptidases as regulators of the plasminogen system. *J Clin Invest* 96, 2534-2538 (1995)
62. Miles, L. A., E. G. Levin, J. Plescia, D. Collen, & E. F. Plow: Plasminogen receptors, urokinase receptors, and their modulation on human endothelial cells. *Blood* 72, 628-635 (1988)
63. Felez, J., L. A. Miles, J. Plescia, & E. F. Plow: Regulation of plasminogen receptor expression on human monocytes and monocytoid cell lines. *J Cell Biol* 111, 1673-1683 (1990)
64. Kim, S.-O., E. F. Plow, & L. A. Miles: Regulation of plasminogen receptor expression on monocytoid cells by beta 1-integrin dependent cellular adherence to extracellular matrix proteins. *J Biol Chem* 271, 23761-23767 (1996)
65. Lu, H., H. Li , S. S. Mirshahi, C. Soria, J. Soria, & S. Menashi: Comparative study of fibrinolytic activity on U937 line after stimulation by interferon gamma, 1,25 dihydroxyvitamin D_3 and their combination. *Thromb Res* 69, 353-359 (1993)
66. Jiang, A., K. Lehti, X. Wang, S. J. Weiss, J. Keski-Oja, & D. Pei: Regulation of membrane-type matrix metalloproteinase 1 activity by dynamin-mediated endocytosis. *Proc Natl Acad Sci U S A* 98, 13693-13698 (2001)
67. Pearse, B. M. & M. S. Robinson: Clathrin, adaptors, and sorting. *Annu Rev Cell Biol* 6, 151-171 (1990)
68. Tabengwa, E. M., L. H. Abou-Agag, R. L. Benza, J. A. Torres, M. L. Aikens, & F. M. Booyse: Ethanol-induced up-regulation of candidate plasminogen receptor annexin II in cultured human endothelial cells. *Alcohol Clin Exp Res* 24, 754-761 (2000)
69. Ploplis, V. A., P. Carmeliet, S. Vazirzadeh, I. Van Vlaenderen, L. Moons, E. F. Plow, & D. Collen: Effects of disruption of the *plasminogen* gene in mice on thrombosis, growth and health. *Circulation* 92, 2585-2593 (1995)
70. Bugge, T. H., M. J. Flick, C. C. Daugherty, & J. L. Degen: Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. *Genes Dev* 9, 794-807 (1995)
71. Ploplis, V. A., E. L. French, P. Carmeliet, D. Collen, & E. F. Plow: Plasminogen deficiency differentially affects recruitment of inflammatory cell populations in mice. *Blood* 91, 2005-2009 (1998)
72. Moons, L., C. Shi, V. Ploplis, E. Plow, E. Haber, D. Collen, & P. Carmeliet: Reduced transplant arteriosclerosis in plasminogen-deficient mice. *J Clin Invest* 102, 1788-1797 (1998)
73. Carmeliet, P., L. Moons, V. Ploplis, E. Plow, & D. Collen: Impaired arterial neointima formation in mice with disruption of the plasminogen gene. *J Clin Invest* 99, 200-208 (1997)
74. Drew, A. F., H. L. Tucker, K. W. Kombrinck, D. I. Simon, T. H. Bugge, & J. L. Degen : Plasminogen is a critical determinant of vascular remodeling in mice. *Circ Res* 87, 133-139 (2000)
75. Busuttil, S. J., C. Drumm, V. A. Ploplis, & E. F. Plow: Endoluminal arterial injury in plasminogen-deficient mice. *J Surg Res* 91, 159-164 (2000)
76. Ling, Q., A. T. Jacovina, A. B. Deora, P. Nahirney, M. Febbraio, R. Simantov, R. L. Silverstein, B. L. Hempstead,

R. T. Hahn, D. A. Fischman, & K. A. Hajjar: Deficiency of the fibrinolytic receptor, annexin II, is associated with deposition of fibrin, defects in thrombolysis, and impaired angiogenesis. *Blood* 98, 788a, (2001)
77. Silverstein, R. L., R. J. Friedlander, Jr., R. L. Nicholas, & R. L. Nachman: Binding of lys-plasminogen to monocytes/macrophages. *J Clin Invest* 82, 1948-1955 (1988)

Key Words: Plasminogen, Plasmin, Receptor Modulation, Urokinase, Plasminogen Activator, Receptors, Plasma Cell Membrane, Tumor, Cancer, Leukemia, Knock-out Mice, Inflammation, Thrombus, Arthritis, Cell Differentiation, Review, Platelets, Thrombocytes, Review

Send correspondence to: Dr. Edward F. Plow, Ph.D., Head of Research, Joseph J. Jacobs Center for Thrombosis and Vascular Biology; Chairman, Department of Molecular Cardiology; Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, Tel: 216-445-8200, Fax: 216-445-8204, E-mail: plowe@ccf.org