Concise Review

Structure, Function and Expression on Blood and Bone Marrow Cells of the Urokinase-Type Plasminogen Activator Receptor, uPAR

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ABSTRACT

Several important functions have been assigned to the receptor for urokinase-type plasminogen activator, uPAR. As implied by the name, uPAR was first identified as a high affinity cellular receptor for urokinase plasminogen activator (uPA). It mediates the binding of the zymogen, pro-uPA, to the plasma membrane where trace amounts of plasmin will initiate a series of events referred to as "reciprocal zymogen activation" where plasmin converts pro-uPA to the active enzyme, uPA, which in turn converts plasma membrane-associated plasminogen to plasmin. This is an efficient machinery to generate broad-spectrum proteolytic activity which is spatially restricted to the plasma membrane, since plasmin that diffuses away from the plasma membrane is rapidly inactivated by circulating inhibitors (i.e., α_2-antiplasmin). The system is controlled by a series of plasminogen activator inhibitors (PAIs), most importantly PAI-1 and PAI-2, providing means of temporally restricting the process of plasminogen activation.

In addition to its role in plasminogen activation, compelling evidence has demonstrated a role for uPAR in cell-cell and cell-extracellular matrix adhesion, both directly and indirectly. uPAR is directly involved in binding to the extracellular matrix molecule, vitronectin, and the affinity of this binding is increased when uPAR is occupied by (pro-)uPA. A more indirect but presumably very important role of uPAR in cell adhesion seems to be mediated through interactions between uPAR and β_1- or β_2-integrins. It has been demonstrated that uPAR may bind physically to integrins in a reversible manner. The interaction seems to be of functional importance since the affinity of the integrin for its corresponding ligand is modulated by the association of integrin with uPAR. In some experimental setups uPAR has been shown to reduce the affinity of the associated integrin for certain ligands, while other experimental systems have demonstrated an increased affinity of the interaction between integrin and ligand after binding of uPAR to the integrin.

Finally, uPAR has also been shown to participate in signal transduction events. Since uPAR is not a transmembrane molecule but belongs to the group of proteins that are tethered to the plasma membrane via a glycosyl-phosphatidylinositol anchor, association with a transmembrane adaptor is required for transmission of signals via uPAR. Integrins may serve as such signal transducers, and indeed uPAR has been shown to be associated in the plasma membrane with complexes of integrins and (phosphorylated) tyrosin kinases suggesting a role for these complexes in transmembrane transmission of signals via uPAR.

In the hematopoietic system it has been shown that urokinase-type plasminogen activator (uPAR) is expressed as a differentiation antigen on cells of the myelomonocytic lineage and as an activation antigen on monocytes and T lymphocytes. Neutrophils contain intracellular reservoirs of uPAR that are translocated to the plasma membrane upon activation, and neutrophils from patients with the rare blood disease paroxysmal nocturnal hemoglobinuria (PNH) that fail to express glycosyl-phosphatidylinositol-anchored proteins including uPAR, show a very significantly reduced transmigration over an endothelial barrier. Cell-associated plasminogen activation by PNH-affected neutrophils is severely impaired, and it has been proposed that this may be causally related to the propensity for thrombosis in PNH. The pattern of expression of uPAR in hematological malignancies mirrors the expression by normal blood and bone marrow counterparts with some exceptions (differentiated myeloid leukemias are positive, undifferentiated myeloid may be negative and the majority of lymphoid leukemias and lymphomas are negative). The potential clinical relevance of uPAR expression in leukemias and lymphomas has not been determined. ***Stem Cells*** *1997;15:398-408*

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Physical Characteristics

The Protein

The receptor for uPAR was first identified on monocytes and the monocytoid cell line U937 as a membrane protein that binds the amino-terminal fragment of urokinase plasminogen activator (uPA) with high affinity (kDa $\approx 5 \times 10^{-10}$ M) [1-3]. uPAR consists of a single polypeptide chain and behaves as an amphophilic membrane protein [3, 4]. Exposure of monocyte-like cells to phorbol myristate acetate (PMA) causes increased expression of uPAR-protein and mRNA, a reduced affinity of uPAR for its ligand and increased molecular heterogeneity of uPAR [3-6]. The mass and charge heterogeneity is probably accounted for by variations in glycosylation. The uPAR molecule (molecular weight [mw] ≈ 55 kDa) has been shown to be heavily glycosylated. The polypeptide backbone of uPAR has an apparent mw of 35 kDa [7]. The NH2-terminal part of the receptor plays a primary role in ligand binding [8-11]. The COOH-terminal part of uPAR is linked to the plasma membrane by a glycosyl-phosphatidylinositol anchor [12-14]. uPAR is a cysteine-rich molecule with a three-domain structure defined by intrachain disulphide bonds. The structure shows homology with members of the Ly-6 family of glycolipid-anchored proteins, and with the secreted, single domain snake venom alpha-neurotoxins [8, 15, 16]. Monoclonal antibodies to uPAR have been assigned the "cluster of differentiation" (CD) No. 87 [17].

The Gene

The cDNA for uPAR has been isolated as a 1.4 kb clone that encodes a protein of 335 amino acids including an NH2-terminal signal sequence of 22 residues [18]. The uPAR gene encodes an NH_2 as well as a COOH-terminal signal sequence [18]. The COOH-terminal part of the nascent protein is removed during the final processing and immediately followed by transfer of the protein to a preformed glycolipid anchor, presumably leaving a protein of 283 amino acids as the mature product [12, 19]. Independent of these observations the cDNA for a previously described monocyte activation antigen, Mo3, was isolated and found to be identical to the uPAR gene [20]. Analysis of the structure of the uPAR gene has revealed seven exons spread over 23 kb of genomic DNA and has further substantiated the relationship of uPAR with other members of the Ly-6 and snake venom alpha-neurotoxin family of proteins [16, 21]. The gene has been traced to a specific region of the long arm of chromosome 19 [22].

Physiological Activity

The Plasminogen System

uPA binds to uPAR by the amino-terminal domain, while the catalytic, carboxyl-terminal domain is not blocked by the binding process (Fig. 1) [23, 24]. The amino-terminal, receptor-binding part of uPA is referred to as the growth factor domain due to sequence homology with epidermal growth factor [23]. This orientation of receptor-bound uPA equips the cell with a potential for plasminogen activation. A strong acceleration of the activation of plasminogen was found with optimal concentrations of pro-uPA in the presence of U937 monocytes as opposed to a cell-free system [25]. The enhanced plasminogen activation by receptor-bound pro-uPA/uPA seems to be due to a combined effect including an increase in the rate of feedback activation of pro-uPA by cell-bound plasmin [25] as well as an increase in the

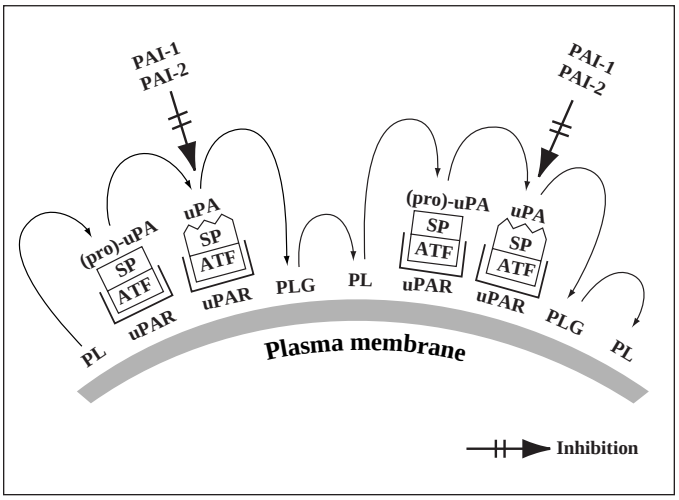

Figure 1. Diagram illustrating the binding to uPAR and subsequent activation of (pro)-uPA on the plasma membrane. *The "growth factor domain" (ATF) of (pro)-uPA is directly involved in the binding to uPAR leaving the catalytic domain (SP) of (pro)-uPA free. By association with uPAR, pro-uPA is approximated to and subsequently activated by trace amounts of plasminogen (PL) bound to the plasma membrane. Enzymatically active uPA will subsequently activate plasminogen (PLG) to generate more plasmin in a process that is referred to as "reciprocal zymogen activation" [27]. Plasmin that diffuses away from the plasma membrane is rapidly inactivated by circulating inhibitors, primarily α_2-antiplasmin [26]. Plasminogen activator inhibitors (e.g., PAI-1 and PAI-2) may directly inhibit uPA and thereby downregulate the activity of the system. The result of this system is efficient generation of broad-spectrum proteolytic activity that is spatially and temporally restricted on the plasma membrane.*

activation of cell-bound plasminogen, catalyzed by uPAR-bound two-chain uPA [26] in a process referred to as "reciprocal zymogen activation" [27]. In this context uPAR seemingly serves as a template for binding and approximation of pro-uPA/uPA to its substrate, plasminogen, on the plasma membrane [28, 29]. In addition to lowering the Km value of uPA-mediated plasminogen activation to a level below the physiological plasminogen concentration of 2 μM, plasmin generated in the cell-surface plasminogen activation system is relatively protected from its principal physiological inhibitor α_2-antiplasmin [26]. Receptor-bound uPA may, however, be inhibited efficiently by specific plasminogen activator inhibitors ([PAI]-1 and PAI-2) [30-32]. Inhibition of receptor-bound uPA by PAI-1 is followed by internalization and degradation of the complex [33, 34]. The process is mediated via the α_2-macroglobulin receptor (α_2MR), which associates with the uPAR-uPA-PAI-1 complex on the plasma membrane, resulting in an increase of the rate of degradation [35-37]. Pro-uPA bound to uPAR seems to be relatively protected from binding to and degradation by α_2MR [38]. The overall effect of this system is an efficient, but spatially and temporally restricted generation of proteolytic activity with broad specificity.

Myelopoiesis

Myeloid precursors develop into mature blood cells in the bone marrow in a complex milieu of stromal cells and extracellular matrix under tight regulation by a multitude of cytokines that provide positive as well as negative signals for proliferation and differentiation. uPA may directly or more often indirectly via the activation of plasmin contribute to the regulation of myelopoiesis (Table 1). Cytokines that have been sequestered in the extracellular matrix or appear as membrane-bound inactive precursors are released, and in some cases proteolytically processed, by plasmin or uPA to yield biologically active products that may act on myeloid precursor cells. Also phospholipases may be important for the release and activation of cytokines (Table 1). Cytokines may on the other hand exert significant influence on the production of components of the uPA system by stromal cells as well as by monocytes and macrophages (Table 2). A model illustrating some aspects of our current level of understanding of the complexity of this system is shown in Figure 2.

Cellular Migration

Membrane-bound uPA has been shown to polarize at areas of focal cell-substratum contact sites and intercellular contact sites [39, 40]. Migrating monocytes redistribute uPAR to the leading edge and thereby focus uPA activity to this region of the plasma membrane [41]. The redistribution of uPAR and uPA to specific sites of the plasma membrane of migrating cells suggests a possible role for uPAR in cell migration and invasion.

The importance of uPA and uPAR for tissue invasion has been studied in a model system where human carcinoma cell lines were inoculated onto the chorioallantoic membrane of chick embryos. Inhibition of the enzymatic activity of uPA, either by means of an anticatalytic anti-uPA antibody or as a consequence of reduced uPA synthesis following treatment of the carcinoma cells with dimethylsulfoxide was found to block infiltration of the chorioallantoic membrane by the human tumor cell line HEp3 [42]. In the same model system, it has been found that invasiveness is dependent on the level of membrane-bound uPA, which may be a result of either autocrine secretion of uPA and expression of uPAR by the same cells or paracrine secretion of uPA by some cells and expression of uPAR by neighboring cells [43, 44]. The paracrine model seems to have a correlate in human cancer where immunohistological evidence for a collaboration between cancer cells and stroma cells in the production of PA components during tissue invasion has been demonstrated [45, 46].

Several studies have pointed to a role of uPA in chemotaxis of neutrophils [47-49], and the chemotactic effect is independent for the enzymatic activity of uPA [48, 50]. uPAR is polarized at the leading edge of monocytes migrating in a chemotactic gradient [41, 50]. Treatment of monocytes with anti-uPAR antibody or antisense oligonucleotide that blocks

Table 1. Release and/or activation of cytokines by plasmin and uPA

Effectors	Target	Product (released and/or activated cytokine)				Comments	Reference
		bFGF	IL-1	TGF-β	HGF/SF		
Plasmin/PIPL-C/PIPL-D	BM stroma	+					[96, 97]
Plasmin	SE stroma	+		+			[98]
Plasmin	Monocytes		+				[99]
CM of LB6 transfectants	BAE cells			+		Effect mediated via uPAR-bd. uPA	[100]
uPA	FB stroma				+		[101]

Table 2. Regulation of the expression/secretion of components of the plasminogen activator system by cytokines

Effector	Target	Product					Comments	Reference
		uPAR	uPA	tPA	PAI-1	PAI-2		
IL-1β	BM stromal fibroblasts	nd	x10	nd	x0.5	x6		[102]
bFGF	BM stromal fibroblasts	nd	x10	↑	→	→		[102]
TGF-β	BM stromal fibroblasts	nd	x300	nd	x2	→		[102]
IFN-γ	U937 monocytes	x2.1	x2.2					[103]
IFN-γ	U937 monocytes		↑*)			↑	*)IFN-γ > TNF-α	[104]
TNF-α	U937 monocytes		↑*)			↑	*)IFN-γ > TNF-α	[104]
TNF-α	U937 monocytes	→	x2.5*)				*)uPAR secreted	[103]
G-CSF	Monocytes, macrophages		→					[105]
GM-CSF	Monocytes, macrophages		↑*)				*)Inhib. by glucocorticoid	[105-107]
M-CSF	Monocytes, macrophages		↑*)				*)Inhib. by glucocorticoid	[105, 107]
M-CSF	Monocytes				↑	↑		[108]
IL-1	Monocytes		↑			↑*)	*)PAI-2 > uPA	[109]
IL-2	Monocytes		↑			↑*)	*)PAI-2 > uPA	[109]
IL-3	Monocytes, macrophages		↑*)				*)Inhib. by glucocorticoid	[105]
IL-4	Monocytes			↑				[110]
TGF-β	Monocytes				↑	↓		[111]
TGF-β1	Monocytes		↑*)				*)Causes PA and release of matrix-bound bFGF	[112]
Glucocorticoid	Monocytes				↑			[111]
LPS	Monocytes				↓	↑		[111]
LPS	Activated monocytes					↑*)	*)Inhib. by IL-4 and glucocorticoid, potentiated by IFN-γ	[113]

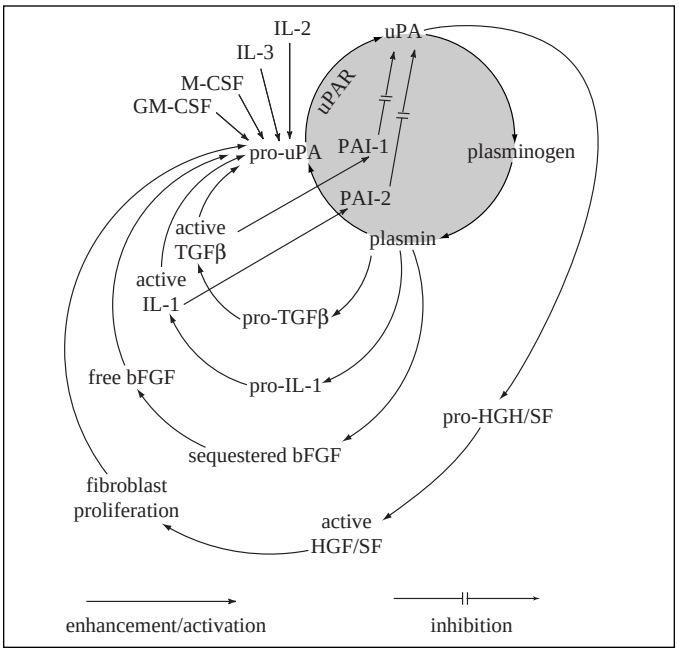

Figure 2. Diagram of the complex interactions between cytokines and the urokinase-type plasminogen activator system. *uPAR binds (pro)-uPA and facilitates its conversion to enzymatically active uPA in the presence of trace amounts of cell-bound plasmin. Activated uPA generates more plasmin from cell-bound plasminogen. The process has been described as "reciprocal zymogen activation" [27]. Plasmin may cause release and/or activation of cytokines that stimulate (pro)-uPA secretion directly or via induction of fibroblast proliferation (TGF-β, IL-1, bFGF, HGF/SF), and in some cases inhibit uPA by inducing the production and release of plasminogen activator inhibitors (PAI-1, PAI-2)(TGF-β, IL-1). A series of cytokines promote (pro)-uPA secretion independent of activation by plasmin (IL-2, IL-3, M-CSF, GM-CSF).*

the synthesis of uPAR ablates the chemotactic response of monocytes to formyl-methionyl-leucyl-phenylalanine (fMLP) and monocyte chemotactic peptide-1 [50]. Chemotaxis of neutrophils towards fMLP is also inhibited by anti-uPAR antibodies, but not by anticatalytic antibodies to uPA [51]. Addition of uPA has no effect on fMLP-induced chemotaxis of neutrophils, but the association of uPAR and CR3 (see below) may play a role since certain low-molecular weight carbohydrates that interfere with the association of uPAR and CR3 inhibit neutrophil chemotaxis [51].

Adhesion and Cell-Stroma Interactions

Recently it has become clear that uPAR is involved in cell-stroma interactions and signal transducing events that are independent of its role in plasminogen activation. Vitronectin found in extracellular matrix has been shown to bind uPAR, in particular when uPAR is associated with its ligand uPA [52-54]. uPAR may also indirectly play an important role in the regulation of cell adhesion and other cellular functions mediated through interactions with β_1- and β_2-integrins. Cocapping as well as coisolation experiments have demonstrated that uPAR may be physically linked to integrins [55-58]. The interaction between uPAR and CR3 seems to depend on a lectin-carbohydrate like interaction, since cocapping may be inhibited specifically by *N*-acetyl-D-glucosamine [55]. During neutrophil activation and polarization uPAR and CR3, from initially being colocalized on resting cells, are redistributed to separate parts of the neutrophil membrane, uPAR to the lamellopodium and CR3 to the uropod, respectively [57].

Transfection to human embryonic kidney 293 cells with uPAR cDNA has been reported to alter the adhesive phenotype of these cells [58]. The affinity for vitronectin was increased and the β_1-integrin-mediated binding to fibronectin was decreased. The ability of uPAR to modulate integrin-mediated adhesion was dependent on the formation of complexes which include uPAR, integrin and, in this particular cell type, also caveolin, and was most prominent when the integrin was expressed in its activated form [58]. In addition disruption of the physical interaction between uPAR and integrin, i.e., by a selected peptide with affinity for uPAR derived from a bacteriophage peptide display library, was found to restore integrin affinity for its ligands. The particular attachment of uPAR by a glycolipid anchor may facilitate the interaction of uPAR with integrins and possibly other transmembrane proteins by conveying to uPAR a high lateral mobility on the plasma membrane [59]. However, the glycolipid anchor per se is not of crucial importance since soluble, recombinant uPAR may modulate integrin function in a manner similar to glycolipid anchored uPAR [58].

The functional activity of soluble recombinant uPAR demonstrated in these experiments may be biologically relevant since uPAR in some cases has been shown to be proteolytically processed by uPA with subsequent release of domain 1 [60]. In fact signal transduction, resulting in activation of intracellular protein tyrosine kinases, was observed after exogenous addition of in vitro cleaved recombinant uPAR to THP-1 cells mediated by uPA via domain 1 of uPAR, and some as yet unidentified transmembrane adaptor has recently been demonstrated [61].

The effect of uPAR on cell adhesion is reflected by a parallel effect on cell migration. In the presence of uPAR, migration onto vitronectin is increased and migration onto fibronectin decreased, regardless of the mode of presentation, whether it is a result of transfection or the addition of exogenous soluble recombinant uPAR [58]. In the human monocytic cell line THP-1, β_2-integrin (CR3)-mediated binding of its complementary ligand, fibrinogen, was inhibited by recombinant soluble uPAR, and this inhibitory activity of uPAR was enhanced after preactivation of the β_2-integrin, paralleling the observations made with regard to the influence of uPAR on β_1-integrin-mediated binding to fibronectin [58].

On monocytes, uPAR and CR3 polarize and interact at the site of contact with fibrinogen [62]. Anti-uPAR antibodies inhibit the binding of PMA-treated monocytes to the ligands for CR3, fibrinogen and key-hole limpet hemocyanin (KLH), but do not significantly inhibit binding to the ligand for β_1-integrin, fibronectin [62]. Specific inhibition of uPAR expression on monocytes by treatment with antisense oligonucleotide significantly reduces binding of monocytes to KLH via CR3, and this effect may be counterbalanced by adding recombinant soluble uPAR, which restores the binding of monocytes to KLH [62]. Blocking uPA production by an antisense oligonucleotide results in increased adhesion to fibrinogen without affecting the binding to fibronectin, while addition of intact uPA, but not the aminoterminal receptor-binding fragment of uPA, results in reduced affinity for fibrinogen [62]. Thus the maximal enhancing effect on the affinity of β_2-integrin for its ligands is provided by unoccupied uPAR in this particular experimental setting. The complexity of the consequences of interactions between uPAR and integrins is further increased by the observation that CR3 in its activated form enhances uPAR-mediated binding to vitronectin [63]. On monocytes the complex of uPAR and β_2-integrin also coimmunoprecipitates tyrosine kinases, suggesting that the complex is involved in signal transduction [56]. In U937 monocytes binding of uPA to uPAR has been shown to induce tyrosine phosphorylation of a 38 kDa protein [64], and neutrophils from healthy individuals are primed for superoxide production when uPA binds to uPAR, while neutrophils from a patient with the leukocyte adhesion defect that lack CR3 are not primed [65].

Although the interpretation of experimental data obtained with different model systems at the present time has led to divergent conclusions in terms of the influence of uPAR-β_2-integrin interaction on the affinity of the binding of the β_2-integrin to its complementary ligands, data have been reported by several groups supporting that physical interaction between uPAR and β_2-integrin does occur. This interaction may be of functional importance in terms of providing a basis for integrin-mediated transmembrane signal transduction after binding of ligands to uPAR on one hand, and on the other hand the interaction between uPAR and β_2-integrin may provide the basis for a complex pattern of mutual modulations of the affinity of receptor-ligand interactions.

uPAR on Hematological Cells and Their Precursors

The possible relevance of uPAR-integrin interactions for binding and release of myeloid precursor cells from the bone marrow to the blood has not been studied. The β_1-integrins very late activation antigen (VLA)-4 and VLA-5 on myeloid precursors have been shown to be involved in the binding of these cells to bone marrow stroma [66]. The very early myeloid precursor identified by the CD34 marker resides in the bone marrow and does not express uPAR [67]. During differentiation along the granulocyte-monocyte pathway, uPAR expression is gradually acquired and the mature cells are subsequently released into the blood [67]. Theoretically uPAR on maturating myeloid cells may interact with, and modify, integrin-ligand interactions and thereby contribute to the release of immature monocytes and granulocytes from the bone marrow into the blood. However, CD34-positive precursor cells that are released from the bone marrow to the blood after cytokine (G-CSF) stimulation do not express uPAR [67]. Thus if uPAR plays a role in the release of mature monocytes and granulocytes from the bone marrow to the blood through uPAR-integrin interactions on myeloid precursors, this is clearly only one of several mechanisms of regulating binding of precursor cells to bone marrow stroma. In "Dexter-type" long-term bone marrow cultures, it has been found that uPAR is expressed not only by resident macrophages, but also by fibroblast-like stromal cells (unpublished data). It is therefore also possible that uPAR expressed by bone marrow stromal cells may interact with and modify the affinity of ligands on these cells for myeloid precursors. This is, however, purely speculative at the present time and should be experimentally pursued.

uPAR is expressed as a differentiation antigen on monocytes and granulocytes with the highest amounts on mature cells [67]. On eosinophils uPAR is expressed in amounts comparable to the level on neutrophils as judged by flow cytometry, while no conclusion has been reached with regard to expression of uPAR on basophils due to the low number of these cells in normal blood [67]. uPAR was first recognized on monocytes and was independently identified as a monocyte activation antigen, Mo3, with higher levels of uPAR expressed on activated compared to resting monocytes as a result of de novo protein synthesis [68-70]. In addition, an intracellular pool of Mo3 has been detected in monocytes [71]. Anti-Mo3 antibody has been shown to block the response of monocytes to migration inhibitory factor [72, 73].

uPAR is not detected on erythrocytes, platelets and resting B and T lymphocytes, while activated T lymphocytes and natural killer cells do express uPAR [67, 74-76]. Activation of T lymphocytes through β_1- or β_2-integrins results in protein kinase C activation, an increase in intracellular cyclic AMP and expression of functionally active uPAR on the plasma membrane [77]. This is correlated with an increased potential for invasion by activated T cells, both in a Matrigel-based assay and into fibrin clots [77, 78]. It has also been shown that activated T lymphocytes present in tumor biopsies express uPAR [77].

Neutrophils have been shown to contain uPAR in intracellular granules and secretory vesicles, and it has been found that stimulation of neutrophils, e.g., by the tripeptide fMLP, causes immediate translocation of uPAR resulting in increased expression of uPAR on the plasma membrane and in an increased potential for binding of (pro)-uPA and plasminogen activation by activated neutrophils [79].

By and large the expression of uPAR in hematological malignancies mirrors its expression on the corresponding normal counterparts.The blast cells of acute myeloid leukemias regularly express uPAR with higher levels expressed on the phenotypically more mature blast cells of the myelomonocytic series [67]. uPAR is not detected on malignant lymphomas of B or T lymphocyte type, but malignant tumors of true histiocytic origin do express uPAR [67]. However, exceptions to this do exist, and expression of uPAR has been demonstrated on blast cells in some cases of acute lymphocytic leukemia [80]. The possible clinical consequences of uPAR expression in hematological malignancies, e.g., excessive bleeding tendency or extramedullary infiltration by leukemic cells, have not been studied in detail. In this respect it may be relevant that the myeloid leukemias of the FAB subtype M4-M5, which are most prone to develop extramedullary disease, also are the subgroups of acute myeloid leukemias that express the highest levels of uPAR [67].

PNH as a Model for the Understanding of uPAR

PNH is a rare blood disorder. The principle clinical manifestations are a chronic intravascular hemolytic anemia with episodes of exacerbation accompanied by hemoglobinuria, a propensity for thrombosis (in particular of the abdominal veins or veins of the central nervous system), attacks of unexplained abdominal pain which may be due to transient occlusion of small veins of the gastrointestinal tract, and usually also some evidence of bone marrow failure manifested by variable degrees of neutropenia and thrombocytopenia. The common denominator of these variable disease manifestations is a failure of blood cells to express proteins that are normally linked to the plasma membrane via a glycolipid anchor [81]. Some of these serve as complement regulatory proteins, e.g., decay accelerating factor ([DAF], CD55) and membrane inhibitor of reactive lysis ([MIRL], CD59). PNH-affected blood cells are therefore more sensitive to trace amounts of activated complement components present in plasma. This causes a reduced life span of erythrocytes (i.e., hemolysis) and activates platelets. Platelet activation may be the most important single

factor predisposing to the formation of thrombi, but the propensity for thrombosis in PNH is probably multifactorial [82]. The underlying genetic defect causing PNH is a somatic mutation in the PIG-A gene of a bone marrow stem cell causing a block in the first step of the synthesis of the glycolipid anchor: the addition of *N*-acetyl-glucosamin to phosphatidylinositol [83, 84]. Erythrocytes, granulocytes, monocytes and platelets are affected in all PNH patients, while the involvement of B and T lymphocytes is more variable, probably reflecting in the individual patient the level of maturation in the stem cell hierarchy of the earliest cell affected by the PIG-A mutation. The failure of PNH-affected cells to express proteins normally anchored by a glycolipid anchor may serve as a basis for a new diagnostic test for PNH using flow cytometry to demonstrate the absence of glycolipid-anchored proteins from PNH cells [85]. Since uPAR belongs to the group of glycolipid-anchored proteins, PNH-affected cells have been shown to lack uPAR on their plasma membrane [13]. The lack of uPAR on PNH-affected neutrophils may thus contribute to the severe impairment which has been demonstrated in transmigration of PNH-affected neutrophil over an endothelial barrier [86]. At the present time it is not clear whether the migratory defect of PNH-affected neutrophils is indeed caused by the failure of these cells to express uPAR and if so, which of the multiple functions of uPAR is most important in this context.

In addition to uPAR, neutrophils have been shown to also contain (pro)-uPA that is translocated when neutrophils are activated [79, 87]. Endogenous (pro)-uPA will occupy only a minor fraction of the uPAR molecules on activated neutrophils, but exogenous (pro)-uPA may be provided by activated endothelial cells [88]; thereby, uPAR may become more completely saturated when neutrophils come into contact with endothelial cells during transmigration. Thus neutrophils may be provided with a full potential for plasminogen activation during transmigration through endothelial cells, but the biological significance of this is not clear.

It may also be of potential importance for transmigration of neutrophils that the degree of occupancy of uPAR by (pro)-uPA may influence the affinity of uPAR for the extracellular matrix molecule vitronectin as described above. Furthermore uPAR may interact with and modify the affinity of integrins for their complementary ligands and thereby influence the outcome of neutrophil transmigration.

PNH-affected neutrophils and monocytes secrete a truncated form of uPAR [14]. This may be of biological importance in view of the recent finding that recombinant soluble uPAR in some cases may substitute for glycolipid-anchored uPAR in terms of modifying the binding of integrins to their complementary ligands [58, 62]. uPAR secreted by PNH-affected neutrophils may possibly substitute in part for the lack of glycolipid-anchored uPAR on such cells. Provided that uPAR exerts its principal influence on neutrophil migration through interaction with intergrins, binding of secreted uPAR to PNH-affected cells would presumably tend to reduce the migratory defect of PNH-affected compared to normal neutrophils. The secretion of uPAR by PNH-affected neutrophils probably accounts for the elevated levels of uPAR found in plasma of PNH patients [89]. The capacity of normal neutrophils to contribute to plasminogen activation and thereby presumably to fibrinolysis is impaired in PNH patients, and it has been proposed that this impairment of a part of the fibrinolytic system may be causally related to the increased propensity for thrombosis in PNH [13]. Locally produced soluble uPAR from PNH-affected neutrophils may well reach biologically significant levels since neutrophils have been shown to accumulate in thrombi [90]. The involvement of neutrophils in thrombolysis has been recognized for many years, and may gain increased attention in the years to come [90-95].

Conclusions

uPAR has been established as a truly multifunctional molecule. It plays a key role in plasminogen activation by binding and facilitating the conversion of the zymogen pro-uPA to enzymatically active uPA. It participates in cell adhesion, directly by binding to vitronectin and indirectly by interacting with and modulating the affinity of integrins for their complementary ligands. Transmembrane signaling resulting in phosphorylation of intracellular tyrosine kinases may be mediated via uPAR through a transmembrane adaptor, e.g., an integrin. Our understanding of the role of uPAR on blood and bone marrow cells is very limited, but plasminogen activation is functionally important for fibrinolysis (e.g., during tissue repair) and may be important also for release and/or activation of cytokines. Interaction of uPAR with extracellular matrix molecules (e.g., vitronectin) or integrins and possibly other molecules is likely to influence cell motility. The possible contribution of expression of uPAR on the malignant cells to the clinical manifestations of hematological malignancies is unknown.

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