The Urokinase Receptor System, A Key Regulator at the Intersection between Inflammation, Immunity, and Coagulation

Mario Del Rosso*, Francesca Margheri, Simona Serratì, Anastasia Chillà, Anna Laurenzana and Gabriella Fibbi

Department of Experimental Pathology and Oncology, University of Florence, Italy, DENOTHE, Center for the study at molecular and clinical level of chronic, degenerative and neoplastic diseases to DEvelop NOvel THEerapies, University of Florence, Italy, Istituto Toscano Tumori - Italy

Abstract: The urokinase plasminogen activator (uPA) and its receptor (uPAR) provide a cell surface integrated multimolecular complex that exerts pleiotropic functions influencing the development of inflammatory, immune, coagulation and fibrinolytic responses. Here we review the evidences indicating a role of the uPA/uPAR system in the regulation of the innate immune system in the inflammation process, of the adaptive immune response, as well as the role of fibrin and fibrin degradation products at the cross-road between coagulation and inflammation. Comparative studies have clearly highlighted the notion that coagulation and immunity are co-regulated and intertwined. The implication is that the vertebrate blood clotting system is evolutionarily by product of the innate immune system, where the blood clotting proteases have diverged from those comprising the complement system. Differences have emerged gradually, as shown by the acquisition of unique protein structures, such as kringle domains and gla (glutammic acid) domains, in order to comply with the increasingly complex vertebrate systems and to defend higher organisms against a range of infections and injuries. Plasminogen activation also controls the formation of complement anaphylotoxins (responsibe for vasodilatation, increase of venular permeability and leukocyte chemotaxis) and of bradykinin (which accounts for vasodilatation, increase of venular permeability and pain) by regulating the plasma contact system. The urokinase plasminogen activator and its cellular receptor, expressed on the surface of human leukocytes, provide a functional unit that, by regulating interaction of leukocytes with extracellular matrix, as well as its degradation, is critical for the migration of leukocytes and for their movement in the damaged tissues.

Keywords: ????????????????.

INTRODUCTION

Over the last three decades many evidences have been provided, indicating a distinction between the functions of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). While tPA has been identified as the main plasminogen activator involved in thrombolysis *in vivo*, uPA and its receptor (uPAR) are critical in the cell-driven degradation of the extracellular matrix (ECM), which is at the basis of cell invasion within surrounding tissues, including the invasion process required in cancer cells spreading, in angiogenesis and in inflammation (for reviews see [1-3]). uPA and tPA, and the glycosylphosphatydilinositol (GPI)-linked uPA receptor (uPAR), are expressed by several blood-borne cells and are up-regulated during infections and in response to inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interpheron-γ (IFN-γ), interleukin-1 (IL-1) and IL-2 [4]. uPA promotes activation of neutrophils, induces discharge of pro-inflammatory mediators from monocytes [4] and has direct cytolytic effects on several bacteria. uPAR facilitates leukocyte adhesion and migration via uPA-dependent and uPA-independent pathways, and activated neutrophils release a chemotactically active form of soluble uPAR, thus amplifying the inflammatory response by exerting a leukocyte call to action. Deficiency of either uPAR or uPA in mice results in reduced leukocyte recruitment and blunted inflammatory response in models of arthritis and pneumonia [5]. Plasmin, which originates from plasminogen activation-dependent cleavage of plasminogen, has multiple substrates, and thereby a large range of biological effects beyond clot lysis. The zymogen plasminogen binds directly on cells and to ECM, positioning plasmin to degrade ECM proteins and activate pro-matrix metalloproteases (MMPs), thereby facilitating leukocyte migration. Actually, plasminogen-deficient mice are resistant to inflammatory stimuli and display a decreased macrophage migration across ECM [6]. uPAR is expressed in resting T cells and undergoes up-regulation following engagement of the T cell receptor (TCR), or activation by phorbol 12-myristate 13-acetate [7, 8]. uPA and uPAR contribute to natural killer (NK) cell invasion through ECM and IL-2 mediates up-regulation of uPA and uPAR in NK cells [9, 10].

*Address correspondence to this author at the Department of Experimental Pathology and Oncology, University of Florence; Tel: ??????????????; Fax: ????????????; Email: delrosso@unifi.it; fibbi@unifi.it

Besides activation by the so-called extrinsic plasminogen activators (uPA and tPA), plasminogen may be activated by the intrinsic activators kallikrein, coagulation factor XIa (FXIa), coagulation factor XIIa (FXIIa), so-named after their role in the intrinsic branch of coagulation [11]. Through this pathway plasminogen activation also controls the formation of complement anaphylotoxins (which stimulate vasodilatation, increase of venular permeability and leukocyte chemotaxis) and of bradykinin (BK) (accouning for vasodilatation, increase of venular permeability and pain) by regulating the plasma contact system [11]. By interaction with uPAR, both FXIIa and cleaved high molecular weight kininogen (HK) regulate cell growth, fibrinolysis and angiogenesis [12-14].

The overwhelming evidence of the interplay between the uPA/uPAR system, the innate and adaptive defense mechanisms and many components of the intrinsic coagulation/fibrinolysis system compels to consider these pathways as completely integrated.

THE INVOLVED MOLECULES AND THEIR MECHANISM OF ACTION

A. uPAR as a Receptor: uPAR Ligands (uPA, FXII, HKa, VN, PAI-1) and the Plasminogen Activation System

The term invasion connotes the ability of cells to cross anatomical barriers separating tissue compartments (basement membranes, ECM, cell junctions). Extracellular proteolytic enzymes (serine proteinases and MMPs), have been implicated in cell invasion [1], the basic idea being that enzyme activity facilitates cell invasion within degraded ECM and basement membranes. The possibility that plasminogen activation may play a pivotal role in this process has been proposed already at the beginning of this century, this idea has greatly increased over the last decades and now there

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is no doubt that uPA-mediated plasminogen activation to plasmin is critical to the invasion process, as shown in many model systems both *in vitro* and *in vivo*. Biochemistry, molecular biology and cell biology of the plasminogen activation system have demonstrated the presence and structural properties of uPAR, which focalizes the uPA activity at the cell membrane, of the main plasminogen activator inhibitors (PAI-1 and PAI-2) and of the interactions of PAI-1 and uPAR with the ECM protein vitronectin (VN) and integrins [2, 4].

uPAR is a glycosylphosphatydilinositol (GPI)-anchored protein composed of three domains (D1, D2 and D3). It binds with several ligands, namely uPA, VN, the activated form of high molecular weight kininogen (HKa), FXII, each one related with modulation of the invasion properties of the cell, some eventuating into activation of plasminogen to plasmin (uPA, FXIIa), plasmin-dependent ECM degradation and MMPs-dependent ECM degradation triggered by plasmin-activation of pro-MMPs, other with promotion of the cell grip required for cell migration (vitronectin), or with inhibition of cell migration (HKa).

Plasminogen is present in extracellular fluids, including plasma, and localizes within fibrin networks through lysine-binding sites present in the noncatalytic region. Such interaction allows plasminogen activation directly on fibrin strands. Cleavage at the Arg_{561}-Val_{562} bond converts the plasminogen single-chain polypeptide zymogen into plasmin, a broad-spectrum serine-protease consisting of two chains held together by a disulphide bond. Plasmin degrades fibrin and almost all the molecules of ECM either directly or by activation of several MMPs zymogens. Plasminogen is activated to plasmin by intrinsic and extrinsic activators.

1. Intrinsic Activators of Plasminogen. uPAR Interaction with factor XII and HKa

Some plasminogen activators (kallikrein, FXIa and FXIIa) are classified as intrinsic after the evidence that they are generated along with activation of the intrinsic blood coagulation (contact) pathway. Among such activators, only FXIIa has been shown to interact with uPAR.

The body has evolved a mechanism allowing to recognize invasion by foreign materials. Tissues undergoing an inflammatory process, as well as many foreign substances, contain negatively-charged surfaces able to recruit and activate the molecules of the intrinsic (contact) coagulation pathway consisting of FXII, prekallikrein (pre-KLK), high molecular weight kininogen (HK) and FXI [15]. Many proteases of the contact system may convert plasminogen to plasmin. This pathway contributes to about 15% of the total fibrinolytic activity in human plasma [9]. Several studies have shown that kallikrein, FXIIa and FXIa can directly activate plasminogen to plasmin [16-19], as shown in Fig. **1**, thus giving origin to many chemical mediators of inflammation. The activities of pro-inflammatory molecules originating from activation of the contact system is shown in Table **1**.

The protease cascade triggered by activation of the contact system begins with interaction of FXII (Hageman factor, HF) with negatively-charged surfaces, which become available to the contact system within damaged tissues undergoing the inflammation process. The contact with negatively charged macromolecules leads to binding and autoactivation of FXII to FXIIa, activation of pre-kallikrein to kallikrein (KLK) by FXIIa, and cleavage of HK by kallikrein, to release the vasoactive peptide bradykinin. Once formed, kallikrein rapidly cleaves unactivated FXII to FXIIa, in a reaction which is kinetically favored over FXII autoactivation. Bacterial endotoxins, sulphated mucopolysaccharides, collagen and misfolded proteins [20] are a few examples of surface initiators of the contact system activation, which leads to direct and indirect production of many inflammation molecules, as shown in Fig. **1** and Table **1** [11, 21]. However, contact activation by the aforementioned negatively-charged macromolecules may be initiated by an alternative pathway on the surface of endothelial cells. Endothelial cells have bimolecular complexes of uPAR-cytokeratin 1 and gC1qR (endothelial receptor for the C1q fraction of the complement system)-cytokeratin 1 at the cell surface, plus free gC1qR and uPAR. FXII binds primarily to endothelial cell uPAR-cytokeratin complex, to single uPAR and to gC1qR. HK, in turn, interacts with gC1qR-cytokeratin 1 complex, by binding gC1qR with domain 5 of its light chain and cytokeratin 1 with domain 3 of its heavy chain. Pre-kallikrein circulates in blood bound to HK (as also FXI does), and is brought in contact with the endothelial surface upon HK interaction with endothelium. All the components of the contact system are thus assembled on the surface of endothelial cells. Activation of pre-kallikrein to kallikrein in such conditions may involve direct activation by slow autoactivation of uPAR-bound, uPAR-cytokeratin 1-bound or gC1qR-bound FXII (which undergoes both auto- or FXIIa-dependent activation), as well as being independent of FXIIa: heat shock protein 90 (Hsp90) or prolylcarboxipeptidase (PRCP) may activate pre-kallikrein within the pre-kallikrein-HK complex (see Fig. **1** for all the alternative pre-kallikrein activation pathways). Hsp 90 is of particular interest, since it is a protein that is upregulated with tissue stress such as hypoxia or during an inflammatory response. Thus, activation of the contact system cascade along the endothelial surface leads to bradykinin generation from HK [21]. HK is a 120-kDa polypeptide consisting of 6 domains (designated D1 to D6), each one endowed with specific functions. HK consists of a heavy chain (D1 through D3) and a light chain (D5 and D6). The two chains are linked by D4, which contains the sequence of bradykinin. After bradykinin release by proteolysis, the cleaved HK, now called activated HK (HKa), contains a heavy chain and a light chain that remain connected by a disulfide bond. Endothelial cells may bind HK, which becomes a substrate for kallikrein-dependent bradykinin generation from HK D4. Bradykinin is a potent vasodilator directly relaxing smooth muscle by releasing prostacyclin (prostaglandin I-2, PGI2), increases capillary permeability by opening tight junctions between endothelial cells and directly stimulates nerve endings causing pain [22].

It has been recently shown that uPAR also functions as a cell-signaling receptor for FXII [23], which results in promotion of the angiogenesis process in endothelial cells by induction of a uPAR-FXII-beta1 integrin complex, activation of the EGF-receptor and subsequent stimulation of ERK1/2 and Akt phosphorylation (Fig. **2**). Because FXIIa is a direct activator of plasminogen, and although the relationships between the binding sites for uPA and FXII in uPAR remain to be elucidated, it is conceivable that FXII, similarly to uPA, may also direct surface-driven plasminogen activation.

On the contrary, HKa has a well recognised anti-angiogenic activity. Its activity in inhibition of endothelial tube formation has been shown to depend on the property of HKa to disrupt uPA-uPAR complex with vitronectin, to inhibit ERK activation and to block internalization and recycling of uPAR, a series of steps that are critical in angiogenesis (Fig. **2**) [13] .

2. Extrinsic Activators of Plasminogen. The uPA-uPAR System

The two dominant extrinsic plasminogen activators in the body are tPA and uPA. Both are serine proteases secreted as single chain zymogens (pro-uPA, or single-chain uPA, scuPA; pro-tPA, or single-chain tPA, sctPA) and activated by a single cleavage yielding two chains connected by a disulphide bond. Such activation is operated by plasmin itself and by several cathepsins and serine proteases [24]. tPA exerts an efficient fibrinolysis because of the presence of tPA-binding sites on fibrin strands, where also plasminogen is localized. tPA-fibrin interaction involves tPA kringle 2, and the catalytic activity of tPA results enhanced upon fibrin binding. tPA-dependent pathway of plasminogen activation is central to the control of fibrinolysis and thrombolysis [25]. uPA is the only extrinsic plasminogen activator interacting with uPAR, thereby activating a cascade of events at the cell surface that include cell movement and

Table 1. Type-alpha-FXIIa Indicates a Proteolysis-derived form of FXII, Also Called Type-alpha-activated Hageman Factor (alpha-HFa), which Derives from Proteolytic Cleavage of the Single-chain Precursor Within a Sequence Held Together by a S-S Bridge. The Resulting Molecule is a Dimer Composed by a COOH-Terminal B light Chain Containing the Catalytic site and by a NH2-terminal A heavy chain. Because of its property to increase vessel permeability, this factor was Originally Named "Vascular Permeability Factor".

Contact System-derived Pro-inflammatory Effect		
Molecule	**Direct Action**	**Indirect Action**
Type αFXIIa	➢ increased vascular ➢ permeability ➢ angiogenesis	activation PK to KAL release BKfrom HK PLG activation to PL→ PL-dependent inflammatory pathway
Type βFXIIa	➢ chemotactic activity	Activation of C1 → chemotacti activity
FXIa		activation PK to KAL PLG activation to PL→ PL-dependent inflammatory pathway
Kallikrein (KAL)	➢ chemotactic activity	release of BK from HK activation of C3 and C5→ chemotactic activity and vasodilatation βFXIIa formation→ activation of C1 activation of pro-uPA to uPA → ECM invasion PLG activation to PL→ PL-dependent inflammatory pathway
Bradikynin	➢ incresed vascular permeability ➢ pain ➢ hypotension ➢ chemotactic activity	prostaglandin production → vasodilatation

invasion, angiogenesis, inflammation [26]. The relative importance of each plasminogen activator *in vivo* in the control of the thrombotic process has been highlighted by studies in mice deficient of plasminogen and in mice single and double deficient in uPA and tPA. Such studies revealed that single uPA or tPA deficiency predisposes to a thrombotic susceptibility, while only double deficient mice exhibit a plasminogen-deficiency-like overt pro-thrombotic state [27]. Although those studies indicated that uPA and tPA can substitute for each other in fibrinolysis, another study which compared the process of mammary gland adipogenesis in mice deficient in plasminogen with that in mice double deficient in uPA and tPA, has clearly indicated the involvement of at least a third plasminogen activator, tentatively identified with plasma kallikrein [28].

The plasminogen activation system is regulated at several levels. Inhibitors of the system include serpins such as the plasminogen activator inhibitor-1 (PAI-1) for uPA, and α_2-antiplasmin for plasmin.

Both plasminogen and the extrinsic activators (uPA, tPA) bind specific cellular receptors, creating an amplification loop of plasminogen activation directly on the cell surface, able to open a path to invasive cells [29]. Three different classes of plasminogen receptors have been identified, able to provide the cell surface with a low-affinity/high-capacity population of pro-invasive molecules [30]. Different cell binding sites for tPA have been described, involved either in tPA clearance in liver cells [31] or in its localization at the surface of the endothelial cell [32] and neuron [33]. The cellular receptor for uPA (uPAR) was first described in U937 monocyte-like cells [34] and still is the issue of a number of studies showing its critical role in the regulation of cell movement, invasion and proliferation [1, 26].

Cell surface-associated plasminogen activation is often described as a multi-protease amplification cascade, occurring on an insoluble substrate, the cell membrane, a feature which presents similarities with the coagulation protease cascade. In the activation of the system, the protease amplification is mainly the product of the high number of plasminogen/plasmin binding sites, that usually overcome uPAR/tPA receptors number of about 1-2 orders of magnitude [29] (Fig. **3**).

3. Vitronectin, PAI-1 and the "Grip-and-go" Model of Cell Invasion

Cellular migration is also intrinsically linked to cellular adhesiveness. Cells require attachment sites in ECM to assemble their cytoskeleton and to initiate membrane protrusions relevant to migration. However, cell-ECM contact sites cannot be too avid, otherwise the cells will be unable to detach and sneak through. Many indications now suggest that the uPAR system is an organizer of cell-ECM contacts and covers the full range of activities required to promote and disrupt cell attachment sites, according to a "grip-and-go" model of cell migration [29]. The identification of vitronectin

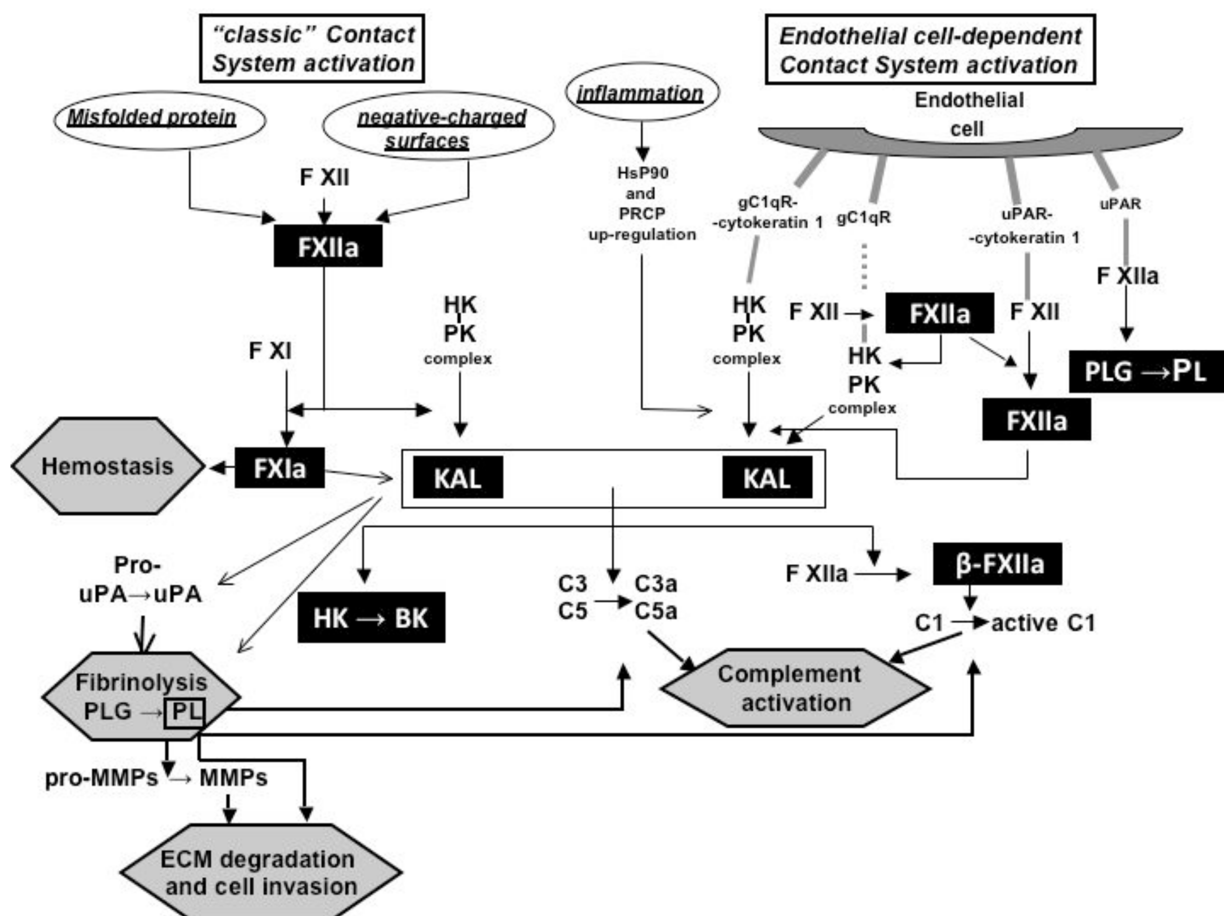

Fig. (1). Factor XII and the generation of chemical mediators of inflammation by the "classic" and "alternative" contact system activation. The left part of the figure shows the "classic" contact system activation, triggered by assembly of the relevant factors on negatively-charged surfaces or on misfolded proteins. The right part of the figure shows endothelial cell-dependent contact system activation, which could be looked-upon as an "alternative" pathway, in analogy to yhe alternative complement activation pathway. Endothelial cells have bimolecular complexes of uPAR-cytokeratin 1 and gC1qR (receptor for the C1 fraction of the complement)-cytokeratin 1 at the cell surface, plus free gC1qR, which is present in molar excess, and free uPAR. Factor XII appears to interact primarily with the uPAR-cytokeratin 1 complex and with free gC1qR. HK (high molecular weight kininogen)-PK (pre-kallikrein) complex binds primarily to gC1qR-cytokeratin 1 complex and to free gC1qR. Activation of the bradykinin-forming pathway can be initiated at the cell surface by gC1qR-induced autoactivation of FXII or direct activation of the PK-HK complex by endothelial cell-derived heat-shock protein 90 (HSP90) or polylcarboxypeptidase that are present and up-regulated within inflammation environment. Whatever its origin, FXIIa activates PK to kallikrein (KALL), giving origin bradykinin (from proteolytic cleavages of HK), complement activation, fibrinolysis. Also the coagulation pathway may be initiated following FXIIa –dependent activation of FXI. It is conceivable that also uPAR-bound FXII may be activated to FXIIa by gC1qR-bound or uPAR-cytokeratin 1-bound FXIIa. On this location FXIIa could have the chance to directly activate PLG to PL or to activate pro-uPA to uPA (intrinsic activation) and to trigger the plasminogen activation cascade. Table **1** shows the pro-inflammation effects of all the main active molecules produced by activation of the contact system.

as an uPAR ligand provided the first evidence of multiple cell migration-promoting properties of uPAR.

uPAR interacts with the ECM protein vitronectin via the somatomedin B (SMB) domain of vitronectin [35, 36]. Binding is mediated by sites located in D2D3 uPAR domains, although an efficient binding to vitronectin requires, as in the case of uPA, the full-length uPAR [37]. Vitronectin binding to uPAR is positively regulated by uPA, likely by inducing the formation of uPAR dimers which show a higher affinity to vitronectin [38]. Since vitronectin is a structural ECM molecule that strongly interacts with integrins by its RGD region (aa 45 to 47), this kind of binding induces on uPAR the gripping properties required for cell migration. Therefore, the uPA/uPAR/ vitronectin /integrin complex may be sufficient to account for all the pleiotropic cellular effects of uPAR (Fig. **3**). However, there are other molecules that modulate this core system (Fig. **3**). PAI-1 also binds vitronectin. Since both binding sites for uPAR and PAI-1 on vitronectin are close to vitronectin SMB domain and are located adjacent to the RGD motif mediating integrin binding, PAI-1 may act as an anti-adhesive factor by preventing uPAR/ vitronectin /integrin interaction [39, 40]. These activities of uPAR and PAI-1 are independent of any proteolytic and anti-proteolytic activity and contribute to the duality of the uPA/uPAR system in cell adhesion and matrix degradation. This scenario is further complicated by the activity of uPAR-bound HKa. The D5 region of HKa binds the somatomedin B domain of vitronectin, thereby masking the vitronectin RGD region and preventing integrin ligation [41]. HKa D5 also directly binds to uPAR D2 and D3, which serves as binding sites for VN [42]. HKa thus competes for vitronectin interaction with both $\alpha v\beta 3$ integrin and uPAR, thereby acting as an antiadhesive factor [43], an activity that results into inhibition of angiogenesis in endothelial cells [13]. Normally, HK shuttles kallikrein, an intrinsic activator of plasminogen to plasmin. Therefore, once bound to uPAR, HKa focuses the enzymatic activity of kallikrein on the cell surface (see above). This pathway of cell

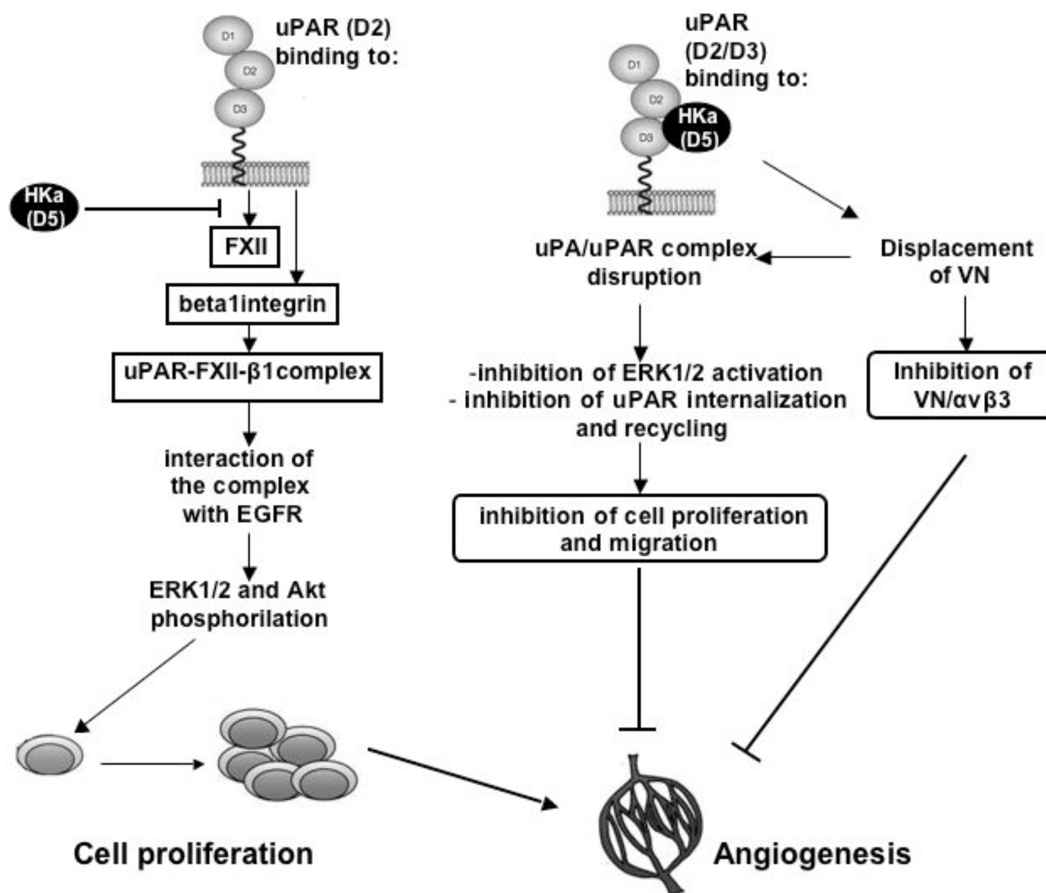

Fig. (2). Opposite activities of factor XII and HKa on uPAR-dependent angiogenesis. The formation of new vessels is a critical step in the evolution of acute to chronic inflammation and in the development of the granulation tissue of chronic inflammation and of the repair process. FXII binds uPAR D2, giving origin to a uPAR-FXII-integrin beta1 complex. The complex interacts wit the receptor for the epidermal growth factor (EGFR), thus phosphorylating ERK1/2 and AKT, which results in endothelial cell proliferation, which is requirr for a proper angiogenesis process. HKa may compete with FXII for uPAR D2 binding, thus impairing the whole process and inhibiting angiogenesis. As shown in the right part of the figure, HKa (domain 5, D5) may also displace vitronectin (VN) from uPAR D2/D3. This event impairs VN interaction with alphav-beta3 integrins, thereby weakening endothelial cell grip to the ECM required for angiogenesis-related migration. Alternatively, HKa-dependent inhibition of angiogenesis may depend on the property of HKa to disrupt uPA-uPAR complex with VN, to inhibit ERK activation and to block internalization and recycling of uPAR, a series of steps that are critical in angiogenesis.

surface-associated plasminogen activation is alternative but not exclusive of the classic activation by uPA. The combined activities of HK and of kallikrein lead to an overall cell-detachment activity of uPAR-associated HKa.

A large body of evidence indicates that uPA, vitronectin, HKa, FXII and uPAR itself deliver into the cell proteolysis-independent signals that account for uPAR-dependent cell adhesion, migration and proliferation [26, 44]. By its chemical nature, the GPI anchor of uPAR, which deepens into the first layer of the surface membrane phospholipid bilayer, is unable to transduce extracellular signals. Nevertheless, uPAR binds to other molecules on the cell surface, provided with cytosolic domains and capable of signal transduction upon uPAR stimulation. As a whole, such molecules may be considered as uPAR receptors [3] and are components of a "uPAR transductome" which is assembled on lipid rafts.

B. uPAR as a Ligand: Integrins, GPCR, EGF-R, The uPAR Transductome

Beta1, beta2, beta3 and beta5 integrins interact with uPAR on the cell membrane [45-49]. Among various integrins, uPAR seems to have the highest affinity to the fibronectin receptors alpha3-beta1 and alpha5-beta1 (Fig. **4**). uPAR domain 3 (D3) seems to be relevant in the interaction [50]. uPAR interaction with integrins mainly occurs in a cis form, but a trans interaction has been described, which induces cell-cell interaction [51]. Regions important for ligand binding by integrins have been mapped to the N-terminal portions of integrin alpha and beta subunits [52]. By mapping the binding site of uPAR on integrin subunits, a surface loop within the beta-propeller of the alpha3 integrin chain has been identified, located immediately outside the laminin-5 binding region [53]. Experimental evidence suggests that the alpha3 integrin beta-propeller is shared by other alpha subunits, since the interaction between uPAR and integrin alpha5 beta1 regulates cell migration on fibronectin [54, 55], alpha5-beta1 signalling [56, 57] and the assembly of a fibronectin matrix. uPAR binding induces a conformational change in alpha5-beta1 integrin, which enhances the strength of cell binding to fibronectin in a RGD-independent fashion [58]. uPAR peptide M25, designed on the basis of the uPAR-binding sequence of the alpha3 integrin beta-propeller, and other peptides that span uPAR-integrin binding region, are able to disrupt uPAR-integrin interaction, independently of the integrin alpha subunit, thereby inhibiting integrin-dependent uPAR signalling [59-61]. Many integrins have been shown to interact with soluble uPAR (suPAR) in its native D1D2D3 form, including beta1 integrins which are unable to interact with the truncated D2D3 form of the receptor [62]. Physical association of uPAR with beta2 integrin was first shown in neuthrophils by co-capping of uPAR and complement receptor type 3 (CR3, CD11b/CD18, Mac-1) [40]. The issue of beta2 integrin-

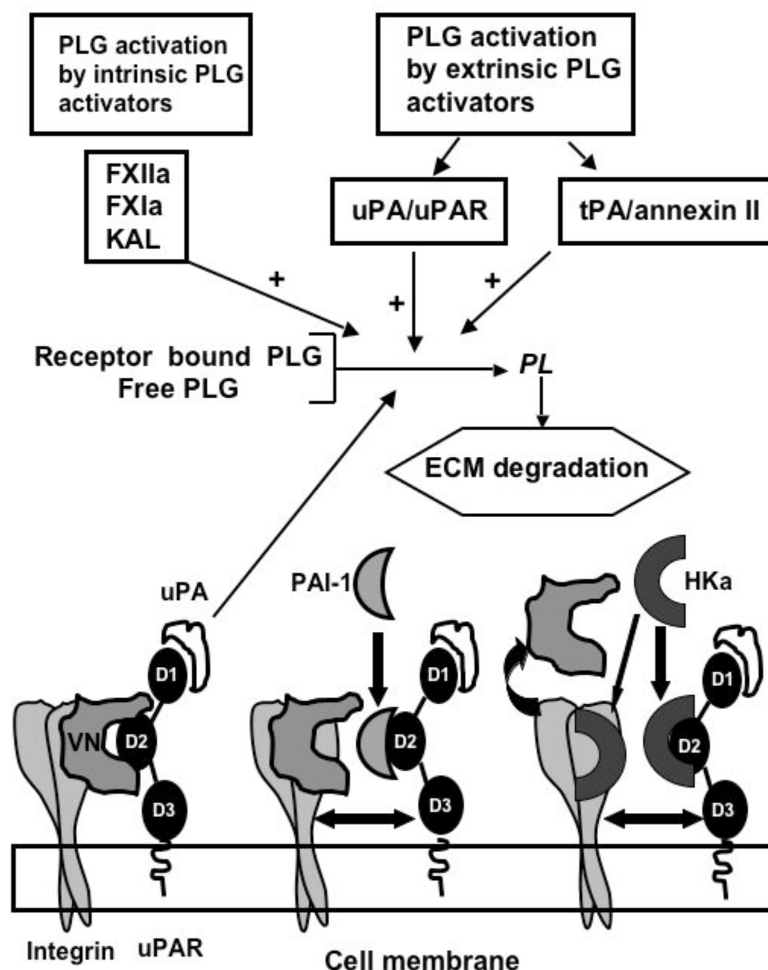

Fig. (3). Plasminogen activation by intrinsic and extrinsic activators. The uPA-uPAR-VN system and its modulation. The upper part of the figure shows PLG activation by intrinsic and extrinsic plasminogen activators. PLG activation by receptor-associated extrinsic plasminogen activators has been recognized to drive cell invasion within tissues following activation of receptor-bound and/or of free PLG. PLG activation by uPA and tPA may be controlled by plasminogen activation inhibitors (PAIs). The possibility of intrinsic PLG activators to activate receptor-bound PLG is not yet a defined issue. This kind of PLG activation is more likely to be important within inflamed tissue, where it gives origin to inflammation chemical mediators. The lower part of the figure shows the basic model of uPA-uPAR system in invasion: Interactions of uPA with D1 of uPAR, of VN with uPAR and with uPAR-bound integrins, provide the functional basis for a "grip-and-go" model of cell invasion. This system may be modulated by PAI-1 and by HKa. PAI-1 binds VN. Since both binding sites for uPAR and PAI-1 on VN are close to VN SMB domain and are located adjacent to the RGD motif mediating integrin binding, PAI-1 may act as an antiadhesive factor by preventing uPAR/VN/integrin interaction. The D5 region of HKa binds the somatomedin B domain of VN, thereby masking the VN RGD region and preventing integrin ligation by VN. HKa D5 also directly binds to uPAR D2 and D3, which serves as binding sites for VN. HKa thus competes for VN interaction with both $\alpha v\beta 3$ integrin and uPAR, thereby acting as an antiadhesive factor, an activity that results into inhibition of angiogenesis in endothelial cells.

uPAR interaction in leukocyte "call to action" in the inflammation process is treated in another section of this review. In human umbilical vein endothelial cells, uPAR forms a signalling complex containing alphav-beta3 or alpha5-beta1, caveolin, and src kinase Yes [63]. uPAR has been shown to associate with beta1 and beta2 integrins of fibrosarcoma cells [48].

Integrins are not the only uPAR receptors that comply with uPAR activities. Other interactors include the G protein-coupled receptor FPRL1 (N-formyl-peptide receptor-like 1), the epidermal growth factor (EGF) receptor (EGFR) and others [26].

fMLP is a pyogenic bacteria-specific formylated peptide that stimulates chemotaxis of polymorphonuclear (PMN) granulocytes by activating seven transmembrane domain G-protein-coupled receptors [3]. The fMLP receptors belong to three different types: the high-affinity N-formyl-peptide receptor (FPR) and its homologues FPR-like 1 (FPRL1) and FPR-like 2 (FPRL2). FPRL2 is unable to bind fMLP, while FPRL1 binds the peptide with lower affinity than FPR [3]. Soluble uPAR (suPAR) may be cleaved by various proteases within the D1-D2 linker region, with the release of D1 and the subsequent exposure of the chemotactic sequence SRSRY (amino acids 88-92) [64-66], while full-length suPAR is unable to bind FPRL-1. The cleaved suPAR, as well as peptides containing the sequence SRSRY, bind FPRL1 in monocytes, thereby stimulating their migration [67]. Full-length GPI-anchored uPAR interacts with FPR through the specific SRSRY sequence [62] and can be considered as an endogenous ligand for fMLP receptors (Fig. **4**) [67, 68], whose expression is required for fMLP-directed migration of monocytes and epithelial cells [62, 69]. However, while chymotrypsin-cleaved soluble uPAR (D2D3[88-274]) elicits migration of monocytic cells through interaction with FPRL-1, it also modulates the ability of monocytes to migrate in response to other chemokines by a decrease of chemokine-induced integrin-dependent rapid cell adhesion [70].

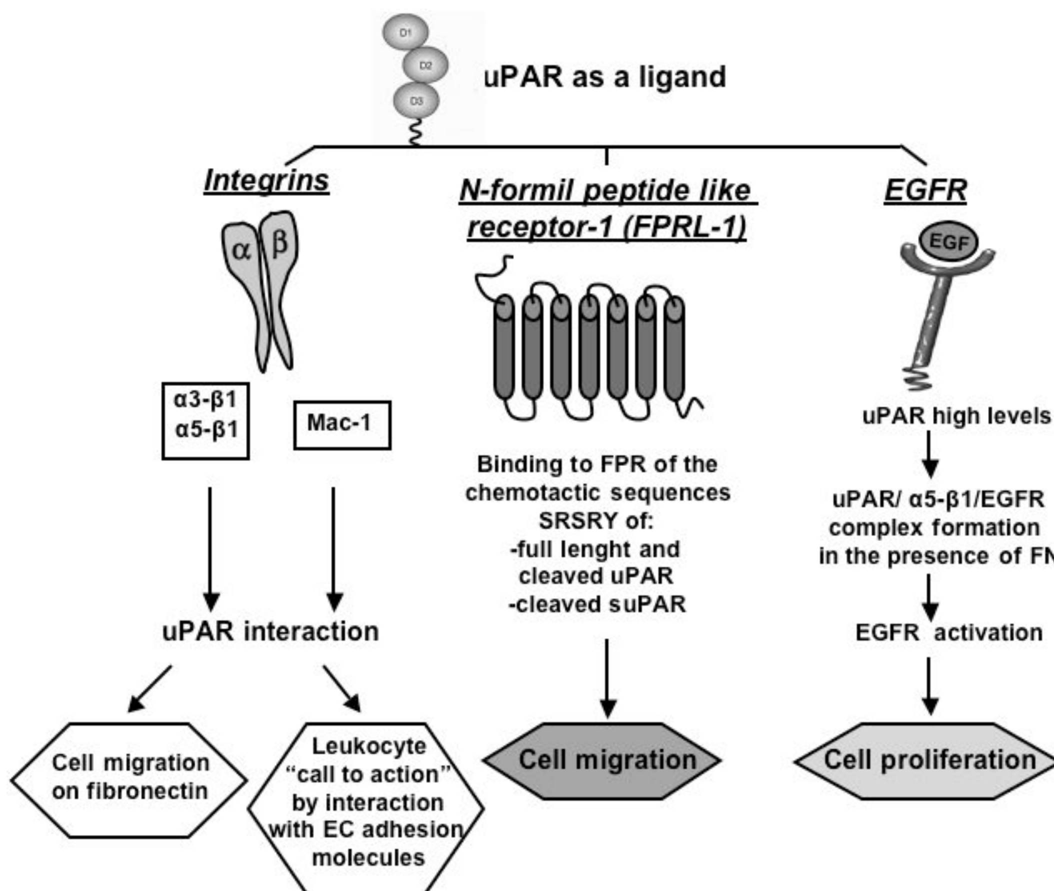

Fig. (4). uPAR as a ligand: pro-inflammation activities of the uPAR-uPAR receptors complexes. This figure shows how uPAR interaction with uPAR receptors on the surface of leukocytes promotes migration and proliferation of cells of the innate immune system. This group of uPAR receptors encompasses a long series of proteins including some for which the structural basis of the interaction is poorly understood. uPAR interacts with a variety of receptor tyrosine kinases, including PDGF receptor, but uPAR/EGFR interaction is the best characterized. It occurs in the presence of high uPAR levels, involves interaction with alpha5-beta1 integrins and the formation of a complex composed by uPAR, alpha5-beta1, EGFR, in the presence of fibronectin (FN). EGFR undergoes phosphorylation which results into cell proliferation. The chemotactic sequence of uPAR (namely SRSRY), expressed by full-length and cleaved uPAR, cleaved suPAR (soluble uPAR), but not by full-length suPAR, binds to N-formil-peptide receptor (FPR), thus inducing cell migration. As shown on the left side of the figure, uPAR interacts with alpha3-beta1 and alpha5-beta1 integrins, increasing the strength of integrin interaction with FN and promoting leukocyte migration on FN. Finally, leukocyte MAC-1 uPAR interaction is the most efficient structural motif related to leukocyte adhesion to endothelial cell adhesion molecules in the process of leukocyte call to action in the inflammation process.

Another uPAR receptor is the EGFR (Fig. **4**). This receptor co-immunoprecipitates with uPAR and is activated by high levels of uPAR, such activation is alpha5-beta1 integrin-dependent and is required for cell proliferation *in vivo* [71].

At present it is not known what is regulating uPAR association with its transmembrane partners. There is the possibility that uPAR associates with various interactors depending on its molecular form. uPAR may be present on the cell surface in at least 3 different forms: a monomer, a dimer or a cleaved molecule [72, 73]. Each form can localize in specific sites of the cell membrane, associate with specific partners and therefore transduce by different signalling pathways, as detailed in another review of this series.

uPA and uPAR in Innate and Adaptive Immunity

Many observations relate the fibrinolytic system to innate and adaptive immunity. uPA and uPAR are expressed by a variety of cells of the hemopoietic lineage [74]. The levels of various molecules of the plasminogen activation system are dysregulated during severe infections, such as those sustaining the so-called "disseminated intravascular coagulation" (DIC). Severe infection and inflammation almost invariably lead to hemostatic abnormalities, ranging from insignificant laboratory changes to severe DIC. Systemic inflammation as a result of severe infection leads to activation of coagulation, due to tissue factor-mediated thrombin generation, downregulation of physiological anticoagulant mechanisms, and inhibition of fibrinolysis. Proinflammatory cytokines (IL-1beta, TNFalpha) play a central role in the differential effects on the coagulation and fibrinolysis pathways [75]. Circulating levels of uPA and its inhibitor PAI-1 change upon bacterial infection. This effects are triggered by bacterial endotoxins and cytokines of the innate immunity, such as IL-1beta and TNF-alpha, which are released by leukocytes upon infection, that elicit expression and secretion of uPA by several cells, such as epithelial and endothelial cells, monocytes and neutrophils [76]. In turn, the local release of uPA contributes to bacterial-dependent neutrophil activation. uPA potentiates neutrophil activation, super-oxide production [77, 78] and migration by either uPAR-dependent [79, 80] or uPAR-independent pathways [77]. uPA-dependent activation of cell-bound PLG to PL enhances the release of pro-inflammatory cytokines and activates the zymogens of MMPs, thereby amplifying the acute inflammation reaction [81]. Additionally, uPA is required for the generation of type 1 immune responses in mice, supporting a role for uPA in the development of protective immunity [82]

1. INNATE IMMUNITY AND INFLAMMATION

The Leukocyte's Call to Action: From Chemotaxis to Phagocytosis

In a previous review [11] we have described the activities of all the molecules originating from the plasminogen activation system in terms of their roles in the redness, heat, swelling and pain (the "cardinal" signs of inflammation). Here we will focus on role of the system in recruitment to an inflammation site of the leukocytes of the innate immune system.

The purpose of inflammation is to convey fluid and cells to a site of injury. Fluid is delivered first, in a matter of seconds. Cells take a little longer (minutes) because they cannot just pour out of the vascular system. Before a leukocyte can capture a bacterium within the tissue spaces, many events have to take place: the leukocyte must be summoned to the site of injury by a proper call (chemotaxis), and switch to a higher metabolic level (activation); afterward it must stick to the endothelial surface (margination), sneak through the endothelium (diapedesis), recognize the intruder and become attached to it (recognition-attachment). The plasminogen activation system plays an important role in many of these steps.

Leukocyte recruitment requires regulation of molecules on the surface of leukocytes themselves and of endothelial cells, in a series of processes where the plasminogen activation system plays a major role. Understanding the role of the system in inflammation requires the convergence of all the previously discussed properties of uPAR as a receptor, as a ligand and as a signalling molecule. In order to enter a site of tissue damage, leukocytes behave like "malignant cells" in the process of extra-vasation and invasion of the underlying tissue. In leukocytes, however, the process is regulated by interaction of uPAR with other leukocyte-specific molecules that modulate uPAR invasive and adhesive functions.

A. Chemotaxis/Activation

To reach an inflamed site, leukocytes obey to the chemical call of chemotactic factors. No call by any of the most important exogenous or endogenous chemotactic factor can be satisfied in the absence of a proper function of the uPAR/uPA/beta2-integrin system, which provides the adhesion/degradation interactions between leukocytes and endothelial cell, leukocytes and extracellular matrix, required to invade inflamed tissues. Chemotactic factors, in turn, may arise from the plasminogen activation system itself. The first observation of a chemotactic activity of the non-proteolytic A chain of uPA on uPAR-expressing cells goes back to 1988 [83], when it was shown that exogenous uPA stimulated chemotaxis of human endothelial cells, independent of its catalytic activity. After that observation, uPA-dependent chamotaxis has become an issue in cancer research and the chemotactic activity of uPA has been described in many cell types, including leukocytes, the main actors of inflammation [84, 85]. The production of uPA in inflamed tissues has been observed mainly in joint inflammatory pathologies and has been extensively reviewed elsewhere [86]. Briefly, many inflammatory cytokines present in the synovial fluid of joints affected by Rheumatoid Arthritis (RA), such as M-CSF, G-CSF, GM-CSF, IL-3, stimulate uPA synthesis in RA inflammatory monocyte/macrophage [87]. Under appropriate stimuli, such as bacterial lipopolysaccharide (LPS), monocytes produce GM-CSF and G-CSF, initiating an autocrine loop which leads to enhanced production of uPA [88]. The same stimuli also induce monocytes to secrete IL-1 and TNFalpha which, in turn, induce production of uPA, GM-CSF and G-CSF from synoviocytes [89] and chondrocytes [88]. Thus, both resident articular cells (synoviocytes and chondrocytes) and inflammatory cells (monocyte/macrophage) are able to produce at the same time cytokines and plasminogen activators, in a sort of amplification cascade which results into an increase of uPA activity in inflamed joints. uPA produced within the sites of inflammation can therefore exert chemotactic activity on circulating leukocytes, in a way similar to all the other chemotactic factors. Basophils circulate in the blood and are able to migrate into tissues at sites of inflammation. uPA has been shown to be a potent chemoattractant for basophils, by acting through exposure of the chemotactic uPAR epitope ($uPAR_{84-95}$), which is an endogenous ligand for FPRL2 and FPRL1 [68]. There is increasing evidence that the uPA/uPAR system plays a role in the chemotaxis of inflammatory cells *in vivo* and *in vitro*. Migration of leukocytes to tissue lesions is impaired in $uPA^{-/-}$ and $uPAR^{-/-}$ mice, resulting in impairment of host defenses, bacterial spread, and death [5, 90, 91]. Chemotaxis of inflammatory cells stimulated by uPA *in vitro* and *in vivo* requires binding to uPAR [64, 92, 93] and the presence of a transmembrane adapter able to transduce the chemotactic stimulus [64, 66].

A new impulse to our understanding of uPAR-dependent chemotaxis has been provided by the discovery of chemotaxis stimulated by uPAR fragments, which rely on their property to interact with FPRL1, the G protein-coupled receptor for fMLP, a topic discussed above. Here it seems noteworthy to underline that excess protease activity present within inflamed tissues (plasminogen activators, matrix metalloproteases, cathepsins, lysosomal enzymes), produced by inflammatory cells themselves or by tissue cells damaged by the inflammatory process, is likely to produce high amounts of cleaved uPAR (with an unmasked chemotactic epitope) able to diffuse from the inflammation site and to stimulate leukocyte recruitment (Fig. **5**).

Basically all the cellular actors involved in inflammation have been shown to undergo uPA-uPAR-dependent chemotaxis (neutrophils, eosinophils, monocytes/macrophages, endothelial cells, fibroblasts, mast cells/basophils, T lymphocytes, B lymphocytes, NK cells).

The origin of chemotactic uPA within inflamed tissues (all the main cells of inflammation and the contact activation system through kallikrein/FXIa/FXIIa/plasmin) (Fig. **5**) again underlines the strict relationship between the plasminogen activation system, inflammation and coagulation.

As detailed in another review of this series, uPA-dependent chemotactic signalling, mediated by different uPAR co-receptors (such as integrins, GPCR, EGF-R), leads to activation of uPAR-expressing cells, which is another pre-requisite for performance of any biological task within the inflammation microenvironment.

B. Margination/Diapedesis

Mononuclear cells of the innate immune system represent a reference standard for uPAR, since the very first demonstration of a *bona fide* receptor for uPA on U937 monocyte-like cells [34]. The first evidence of a possible role of uPAR in leukocyte migration was obtained following U937 induction of chemotaxis with fMLP, the pyogenic bacteria-specific formylated peptide that stimulates chemotaxis of polymorphonuclear (PMN) granulocytes by activating seven transmembrane domain G-protein-coupled receptors [3]: such stimulation resulted in clustering of uPAR at the leading edge of migrating cells [94]. Integrins of the beta2 family are the main integrin members on the surface of human leukocytes. Physical association of uPAR with beta2 integrin was first shown in resting polymorhonuclear granulocytes by co-capping of uPAR and complement receptor type 3 (CR3, alphaM-beta2 integrin, CD11b/CD18, Mac-1) [45]. Following migration-related cell polarization, uPAR and Mac-1 dissociate, since uPAR accumulates at lamellipodia and MAC-1 in uropods [95]. uPAR and beta2 integrins have been shown associated with Src signalling molecules in large receptor complexes in mononuclear cells [96]. Mac-1 is the main leukocyte molecule which interacts with the endothelial adhesion molecules ICAM-1 and ICAM-2 in the so called "integrin phase" of leukocyte attachment to endothelium thus triggering PMN and monocytes margination on endothelium at sites of inflammation. Fibrinogen, which is present on the endothelial cell surface [97] as well as within inflamed tissues as a result of vessel leakage, is another

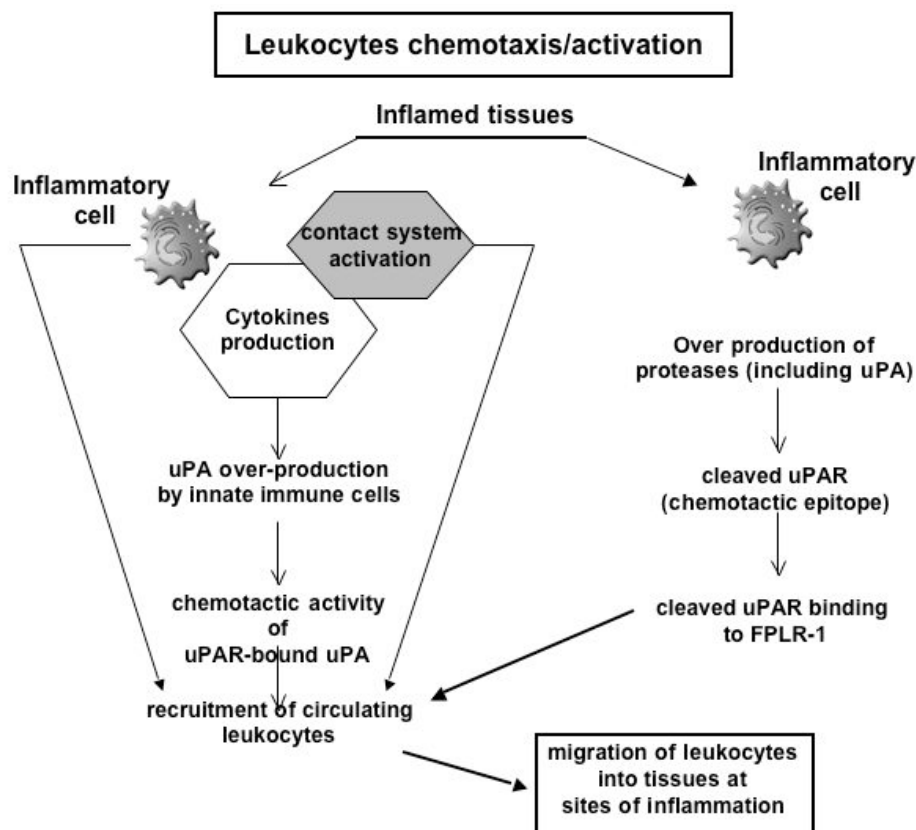

Fig. (5). uPAR and activation of chemotaxis in the inflammatory process. The main endogenous substances exerting a chemotactic stimulus on the leukocyte take origin within the inflammation sites. Chemotactic cytokines and chemical chemoattractants are produced by inflammatory cells and by activation of the contact system, respectively, within the inflammation microenvironment. Cytokines induce uPA over-production by resident cells (i.e. synovial cells in the arthritic joint) and by cells of the innate immune system. The chemotactic activity of uPA and/or of its amino-terminal fragment (ATF), not containing the catalytic site, stimulate chemotaxis and recruitment of uPAR-expressing leukocytes. The sites of inflammation are a forge of proteases (including uPA). uPA and many other proteases (such as metalloproteases, cathepsins, ecc) cleave uPAR thereby exposing its chemotactic epitope. Cleaved suPAR diffuses from the sites of tissue damage and binds to FPR of leukocytes, thereby inducing their migration to sites of inflammation.

preferential substrate for Mac-1, providing an adhesion-migration substrate for the leukocyte. On the surface of monocytes uPAR and Mac-1 form a functional unit whose adhesive function to fibrinogen may be modulated by uPA and vitronectin. uPAR association with Mac-1 enhances the adhesive function of Mac-1, whereas uPAR occupancy by uPA weakens cell adhesion [98]. TGF-beta1 and vitamin D3 up-regulate uPAR and Mac-1 expression on human monocytes, enhancing uPAR affinity for vitronectin, which is further strengthened by Mac-1-fibrinogen interaction. On the other side, uPAR/VN interaction promotes Mac-1-mediated fibrinogen degradation which is carried out by uPAR-bound uPA/plasmin cascade. The other uPAR ligand, uPA, inhibits Mac-1-dependent fibrinogen binding and degradation [99]. This is an example of a finely-tuned molecular system that may alternatively regulate the gripping and the degradation properties of the same cell (Fig. **6**), in a series of alternating cycles of adhesion and invasion ("grip-and-go") that eventuate in cell migration within tissues. Aminoacids 424-440 (peptide M25) of the alphaM subunit (CD11b) of Mac-1 define a region capable of interacting with uPAR. Such a peptide is widely used to disrupt uPAR-beta2 integrin association, which results in impairment of beta2 integrin activity, thus providing the evidence of a positive role of uPAR in the modulation of integrin functions [100].

The ligand binding I-domain of Mac-1 alphaM chain directly interacts with uPA, a binding that impairs integrin functions [98, 101]. The binding of uPA with uPAR and Mac-1 may be simultaneous, since different binding sequences are involved: while the growth factor domain (GFD) of the A chain of uPA interacts with uPAR, the kringle (K) domain of the A chain and the proteolytic domain of the B chain interact with Mac-1. Mac-1, in turn, may simultaneously bind uPA and uPAR by I-domain and non I-domains, respectively. Taken together, uPA and uPAR binding properties of Mac-1 provide a further example of the "grip and go" properties of the plasminogen activation system, based on adhesion, migration and fibrinolysis, exploited by leukocytes to reach an inflamed site [101, 102]. The critical role of uPAR in regulating neutrophil recruitment in inflamed sites has been shown *in vivo* [103]. Similar results have been obtained in the evaluation of uPAR-dependent beta2 integrin activity: the beta2 integrin-dependent recruitment of leukocytes to inflamed peritoneum is reduced in uPAR- deficient mice, whose leukocytes are unable to adhere to endothelial cells [79]. The recruitment of neutrophils to the lung in response to *Pseudomonas aeruginosa* infection, which calls to action leukocytes into the pulmonary parenchyma by a beta2-dependent mechanism, also requires uPAR [90].

It has also been observed that a specific sequence of domain 5 of HK and HKa interact with Mac-1, thus blocking Mac-1-dependent leukocyte adhesion to fibrinogen and to endothelial cells *in vitro* and interfering with neutrophil migration during acute inflammation *in vivo* [43, 104]. The described anti-inflammatory properties of HK are independent of uPAR and identify HK as a controller of cell adhesion balance by preventing excessive leukocyte recruitment and hyperinflammatory responses (Fig. **6**).

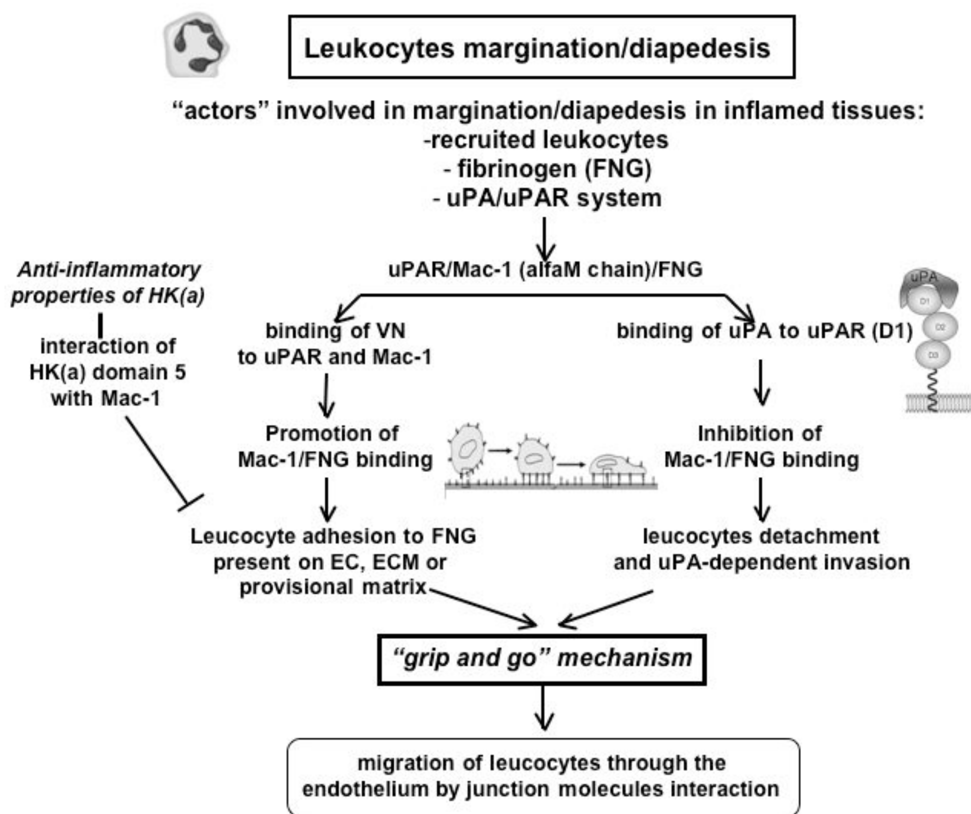

Fig. (6). The role of uPAR, fibrinogen, integrins and HKa in leukocyte margination. This is a typical mechanism that connotes the "grip-and-go" features of leukocyte recruitment within inflamed tissues. Leukocytes are circulating at high speed, and can perceive the call of chemotactic substances for only a few seconds while flowing through the microcirculation. Therefore, a mechanism allowing them to stick to the walls of microvessels in order to perceive chemoattractants call is set-up at the endothelial wall level. The sticking system involves leukocytes, fibrinogen (FNG), uPA/uPAR, VN, MAC-1. This model, that strictly comply with experimental data, involves binding of endothelial cell-associated FNG to uPAR-bound Mac-1 alpha M chain. The simultaneous binding of VN to uPAR and Mac-1 increases the stability of Mac-1-FNG interaction, thus stabilizing leukocyte adhesion to EC, ECM, and/or provisional matrix. Overall, these events connote the leukocyte "grip". This multi-molecular adhesion complex may be de-stabilized by HK(a) D5 interaction with Mac-1, an event which accounts for the anti-inflammation properties of HK(a). On the other side, binding of uPA to uPAR D1 inhibits Mac-1/FNG binding, inducing leukocyte detachment and uPA-dependent matrix degradation which opens a path to the invading leukocyte.

Finally, the finding that PAI-1-knockout or plasminogen-knockout mice showed optimal inflammatory cell migration and host defense during *Pneumococcal pneumoniae* infection [105] indicates that uPA and uPAR favour innate immune responses mostly by promoting inflammatory cell activation and migration rather than by their fibrinolytic function.

The reported observations on the role of uPAR-integrin complex in leukocyte margination do not seem to hold true in diapedesis. Apparently this event, which occurs in the venular compartment of the micro-circulation, does not occur between the endothelial gaps previously induced by permeability-increasing mediators. Early ultrastructural studies indicated that leukocyte digs its own way out by producing a hole in the endothelial cell [106], in a process that develops according to unknown mechanisms. Given the convoluted nature of the junctions and of the cell membrane, it has been suggested that this kind of "transcytosis" is more apparent than real, based upon the section plane. Many studies consistently have shown that during diapedesis leukocytes squeeze between tightly opposed endothelial cells [107]. It is now generally accepted that leukocyte trans-endothelial migration occurs at the junction, but the leukocyte property to cross a continuous endothelial layer still remains a defined possibility. Whatever the case, many studies indicated that there is no requirement for proteinase-mediated digestion of junctional proteins, although there are indication to the contrary in the mechanisms involved in opening of the blood-brain barrier [108]. On the basis of several independent reports [109], it is suggested that junctional molecules (such as CD31 and cadherins) promote haptotactic (i.e. along a gradient of solid-phase-bound molecules) migration of leukocytes, providing a sort of "molecular zipper" made by molecules on opposed endothelial cells [107]. Once the leukocytes have crossed the endothelium they runt into an obstacle: the basement membrane. Many are deflected by it and squeeze laterally into the narrow subendothelial space, where they stop for about 30 minutes [110] in a sort of stand-by state. Eventually, they burst out into the extravascular spaces, both by sheer violence and by using basement membrane-degrading enzymes (collagenases, elastase, heparanase, uPA, cathepsins, transmembrane leukocyte proteinases, ADAM proteases, ecc) [107] that leave the basement membranes in shambles [111]. The whole process of diapedesis takes no longer than 10 minutes. As soon as the leukocytes are out of the venule, they crawl toward their target by hauling themselves along collagen fibers or other tissue structures.

C. Recognition/Attachment/Phagocytosis

After the leukocyte has been attracted into the extravascular space, it has to identify what to attack and then stick to the target. The clue to recognition must be subtle cell surface differences, including charge, hydrophilic properties, and molecular structure. The recognition of foreign molecular structures is rapid and efficient. Such innate cellular response is mediated primarily by phagocytic macrophages (and antigen-presenting cells in adaptive immunity),

which rely on the recognition of conserved structures on pathogens, pathogen-associated molecular patterns (PAMPs), through pathogen recognition receptors (PRRs). PAMPs include, for example, LPS, lipoproteins, peptidoglycans, heat shock proteins, and oligosaccharides. Many families of PRRs exist, that may be cell-associated (such as Toll-like receptors [TLRs], complement receptors) or soluble. TLRs are the best characterized and are expressed on cells of the innate immunity, on endothelial cells and on platelets [112]. PRRs also recognize host cell-derived factors, as dangerous signals (alarmins) that are generated during infection, inflammation, tissue damage or stress [113]. PAMPs and alarmins are referred to as danger-associated molecular patterns (DAMPs). It is worth to underline that DAMPs are actually included among chemokine-like functions (CLF) chemokines [114]. To day the main difference between DAMPs and CLF chemokines is that DAMP mediate signals of damages (so-called "danger signals") only, whereas CLF chemokines have a broader definition serving as very early extracellular messengers (VEEMs) to signal not only damages but all kinds of cellular events to the surrounding tissues [114]. When PRRs are engaged by DAMPs ligation cells of the innate immune system release pro-inflammation and anti-microbial cytokines and chemokines and up-regulate leukocyte adhesion molecules. These effects may eventuate in the resolution of the insult or in the initiation of the inflammatory response. Altogether, these mechanisme are known as non-opsonic phagocytosis (Fig. **7**). Another mechanism (opsonic or opsonin-dependent phagocytosis) requires serum components, opsonins, that bind to microorganism surface and allow the attachment and internalization of the microorganism. The major opsonins are immunoglobulin G (IgG) and complement fragments. CR3 (complement receptor type-3) is the main phagocytic receptor for particles opsonised with the complement fragment C3b and plays a major role in binding and internalization of C3b-dependent phagocytosis [115]. *In vitro* and *in vivo* studies have shown that uPAR plays an essential role in bacterial defence and phagocytosis. Mice deficient in uPAR (uPAR-/-) showed markedly diminished resistance to bacterial infections compared with wild-type mice [103, 116-118]. The decreased ability of uPAR-/- mice for pathogen clearance *in vivo* has been associated with impaired phagocytic capacity of uPAR -/- neutrophils *in vitro*, suggesting that uPAR is required for an efficient phagocytosis. Recently it has been shown that on the surface of neutrophils uPAR associates in *cis* with complement receptor 3 (CR3) (Fig. **7**). Such an association is indispensable for CR3-mediated attachment of C3b-opsonized organisms, as shown by the reduction of internalization upon removal of cell-bound uPAR by phosphatidylinositol-specific phospholipase C treatment [119]. These results, showing a cooperation between uPAR and CR3 in phagocytosis, are consistent with previous reports showing the regulatory role of uPAR over CR3 functions [79, 80, 98, 99]. The complex between uPAR and CR3 presumably occurs via a polysaccharide moiety of uPAR and lectin domain of CR3 [45, 120]. Upon uPA binding to uPAR, the receptor binds to the region of CD11b located between the I-domain and the divalent cation-binding repeats [80], and uPAR-bound uPA interacts with I-domain of CD11b [101]. This secondary interaction negatively regulate binding of of other I-domain ligands to CR3. Other studies indicate that the adhesive functions of CR3 are strongly dependent on the association of uPAR with uPA [98, 99]. Also CR3 affinity to C3b is regulated by uPAR occupancy with uPA [119].

2. INNATE IMMUNITY AND NATURAL KILLER (NK) CELLS

The discovery of the existence of a cell population with natural cytotoxic activity goes back to the seventies', with the description of a cell population showing a non specific lytic activity on tumor cells [121]. Further studies identified the cell responsible for this activity as a large lymphocyte (diameter of 12-20 μm), provided with abundant granules within its cytoplasm. Hence the name "natural killer" (NK) or "large granular lymphocyte" (LGL). NK cells originate and differentiate from a hemopoietic precursor, possibly stimulated by IL-2, and continue to differentiate once in the blood until reaching the state of large non-proliferating cells that colonize various tissues or remain in the blood (10-20% of total circulating lymphocytes). Their functions range from the control of viral infections and tumor cell growth to immunoregulation through cytokine production. NK cells are able to perform the lysis of tumor cells and virus-infected cells (or cells infected by other intracellular parasites, such as Mycobacterium Tuberculosis) by engagement of a non specific recognition mechanism, obeying to a sort of "default" program, not MHC restricted, and thereby called "natural". NK target cell recognition may occur through the Fc receptor (CD16) for target cell-bound IgG (antibody-dependent cell-mediated cytotoxicity, ADCC), or through the complex of KIR (killer cells immunoglobulin-like receptors), able to recognize different allelic forms of class I MHC and to inhibit the killer function [122].

Communication between lymphoid tissues and the rest of the body is maintained by a pool of recirculating lymphocytes which pass from the blood into the lymph nodes, spleen and other tissues and back to the blood by the major lymphatic channels. NK cells are a major component of such a pool and are considered as the main performers of the so called "immunological surveillance", so that altered cells expressing neo-antigen on their surface (tumor cells or infected cells) could be identified and summarily eliminated [123]. On these basis, NK cells have been the first lymphocytes to be studied for the presence on their surface of invasion systems enabling them to efficiently sneak within tissue spaces and thereby perform the body surveillance. Therefore, NK cells have been studied for the presence of the cell-associated PA system. uPAR is absent or found only in a minor subset of freshly purified T (NK) cells [124]. However, culturing of lymphocytes in the presence of IL-2 resulted in population of largely (50 to 80%) uPAR-bearing NK cells [124]. Other observations have shown that NK cells also produce uPA and that the uPAR-bound uPA-triggered protease cascade is essential for ECM degradation by invading NK cells [9]. A cooperation of uPA and MMPs in NK cell invasion has also been described [125].

NK cells treated with high concentrations of IL-2 differentiate into lymphokine-activated killer cells (LAK), whose killer efficiency is higher than that of resting NK cells. In agreement with a previous observation indicating that uPAR may be considered an activation antigen in cytokine-stimulated human T lymphocytes [7], IL-2 has been shown to up-regulate both uPA and uPAR in NK cells [10]. In this study, uPA and uPAR mRNA binding proteins (mRNABPs), previously shown to destabilize uPAR mRNA, have been detected in NK cells: following IL-2 stimulation, a down-regulation of uPAR mRNABPs and a reciprocal induction of uPAR mRNA takes place, along with a transcriptional regulation of uPA. These data suggest that IL-2 upregulates both uPA and uPAR in NK cells by post-transcriptional and transcriptional mechanisms, explaining increases in NK cell invasiveness following IL-2 stimulation. uPAR is associated with the alpha v integrin on the surface of human NK cells [126]. Such uPAR-integrin interaction activates an integrin-dependent MAP kinase signalling cascade, suggesting that signalling initiated by uPA binding may depend on the physical association of uPAR with integrins, a process that may be a prerequisite for NK cell accumulation within established tumor metastases during adoptive therapy. Additionally, uPAR cross-linking in an NK cell line increases integrin surface expression by the MAP kinase/ERK ½ signalling pathway [127].

A sub-population of NK cells plays an important role during pregnancy. Such cells are indeed the most represented lymphocyte population in uterine decidual tissue. Lack of NK cells in knockout mice induces intrauterine foetus death, which may be prevented by active NK cells transfer. NK cells seem to promote embryo implant and to maintain uterus integrity during pregnancy. Regulation of

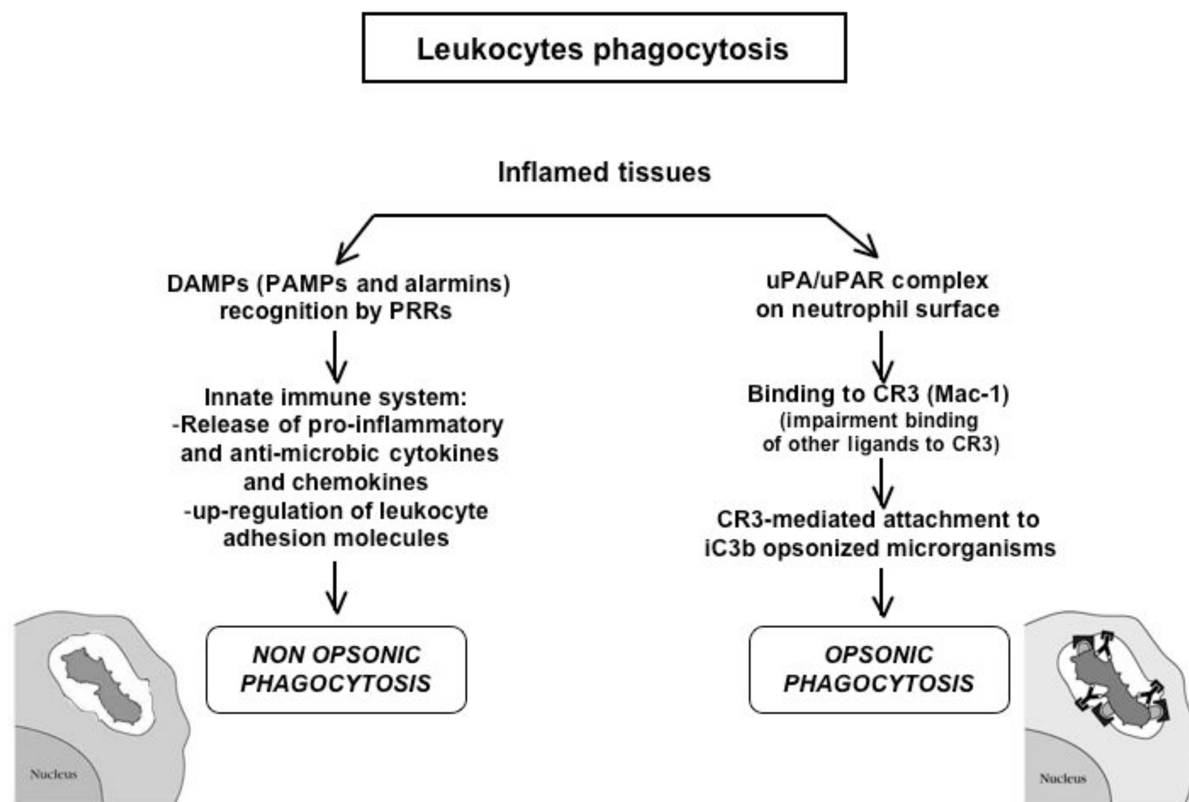

Fig. (7). Role of uPAR in opsonic phagocytosis. As shown in the left part of the figure, pathogens and/or molecules produced within the inflammation environment, connote a series of pathogen-associated molecular patterns (PAMPs), and alarmins, that are referred to as DAMPs (danger-associated molecular patterns), that are recognized by the "default" pattern-recognition receptors (PRRs) of the innate immune system cells. Upon ligand-receptor interaction the leukocyte releases pro-inflammatory cytokines that up-regulate leukocyte adhesion molecules that promote the so-called "non-opsonic" phagocytosis. Alternatively, the uPA/uPAR complex on leukocyte surface binds to the receptor for complement fraction 3 (CR3, Mac-1), impairing at the same time interaction of CR3 with other ligands. In this case, the CR3-mediated attachment of iC3b-opsonized pathogens connotes the so-called "opsonic" phagocytosis, which is an uPA/uPAR-dependent effect.

extravillous trophoblast cell (EVT) invasion of decidua and inner third of the myometrium is critical for a successful pregnancy. It has been shown that uterine NK cells are a source of interpheron-gamma (IFN-gamma) within human early pregnancy decidua. Mechanisms of IFN-gamma inhibition of EVT invasion include both increased EVT apoptosis and reduced levels of uPA and gelatinases [128]. At the same time, uterine NK cells may up-regulate EVT secretion of uPA and gelatinases by an IL-8-mediated pathway [129]. Therefore, uterine NK cells seem to be able to produce a different composition of cytokines that regulate EVT invasion of decidua by controlling the production of uPA and other proteases. Uterine NK cells have been shown to be the major source of EVT uPA, which preferentially associates with NK uPAR [130].

Although the role of NK cells in allograft rejection if far from being understood, a recent study on engraftment and expansion of human hepatocytes in the severe combined immunodeficiency/ albumin linked-urokinase type plasminogen activator (SCID/Alb-uPA) human liver chimeric mouse model supports a critical role for mouse NK cells uPA-dependent rejection of human hepatocytes [131].

An interesting role of NK cell-produced uPA in induction of tolerance associated with eye has recently been shown. In a model of peripheral tolerance called "anterior chamber-associated immune deviation" (ACAID), the differentiation of the T-regulatory suppressor cells depends on NK cells: uPA produced by NK cell binds to uPAR thereby activating plasminogen to plasmin, which in turn activates latent TGF-beta which promotes differentiation of T-regulatory lymphocytes. uPA knockout mice do not develop peripheral tolerance or T regulatory cells. In conclusion, uPA produced by NK cells is required to induce peripheral tolerance in the eye [132].

Finally, a "divergent" receptor for uPA has been described on the surface of mouse NK cells, that is able to inhibit NK function [133]. Such a receptor, namely Ly49E, belongs to the family of inhibiting NK receptors and displays many unique features, among which the property to bind uPA. Upon uPA binding Ly49E delivers into the NK cell an inhibitory signal. Therefore, in addition to uPAR, Ly49E could be involved in uPA-mediated immunologic processes and in tumor biology.

3. ADAPTIVE IMMUNITY

Many lines of evidence indicate that, in addition to modulation of the innate immune response through migration of PMNs and monocytes/macrophages during the cellular phase of the acute inflammation process, uPA and uPAR also participate in the initiation of adaptive immune responses. Migration of activated uPAR-overexpressing macrophages into sites of tissue damage is essential for resolution of acute inflammation and initiation of adaptive immunity, where they behave as antigen presenting cells (APCs), together with resident cells of the same embryonic origin, the dendritic cells (DCs) which, in turn, express uPAR [74]. Also native T cells express uPA and uPAR at low levels, which are rapidly up-regulated during T cell activation [7, 8]. Therefore, the presence of uPA and uPAR in T cells and APCs, has stimulated the hypothesis

that the PA system may have a role in T cell priming. Both *in vitro* and *in vivo* evidences support this hypothesis. Although uPA is a weak mitogen for T cells [134], splenocytes from uPA-deficient mice stimulate only weak T cell activation and proliferation *in vitro* [135], and uPA-deficient mice fail to generate both type 1 and type 2 immune response following pathogen antigen challenge *in vivo* [82, 136]. Blunting uPAR expression on the surface of DCs and monocytes reduces the co-stimulatory capabilities of these cells [137, 138]. Altogether, these evidences demonstrate that mice deficient in uPA have profoundly impaired immunity, involving both Th1 and Th2 polarization, and are largely immunologically unresponsive. Both uPA and tPA are involved in the immunomodulatory actions occurring in liver repair, as observed using carbon-tetrachloride-induced hepatic fibrosis in wild type (WT), tPA-/-, uPA-/- and uPAR-/- mice [139]. Carbon-tetrachloride treatment increased fibrosis in all groups, but significantly less in the three knock-out models, which also showed lack of elevation of serum cytokines and of intra-hepatic T cells, in particular of CD8 subset. Based on these data, plasminogen activators have been postulated to affect fibrosis in part by liver activation of CD8 subsets that govern the fibrogenic activity of hepatic stellate cells.

In the site of infection the presence of a T cell-associated uPA-uPAR system could favour the effector functions of T cells in many ways, such as activation of pro-inflammatory cytokines (pro-TGF beta, pro-IL-1, pro-IL-6) and of pro-MMPs, thereby potentiating the local inflammation response [81].

uPA-dependent fibrinolysis and the associated protease cascade may contribute to the hyper-proteolytic inflammation microenvironment, thus favouring antigen processing and presentation and *in situ* T-cell reactivation [4].

Finally, the importance of uPAR function in blood-tissues recirculation and tissue recruitment previously described for NK cells, has been recognized also in activated T-lymphocytes of the adaptive immunity. Indeed, *in vivo* T cell recruitment to the lung is defective in uPAR knockout mice [91].

FIBRIN AT THE CROSS-ROAD BETWEEN COAGULATION, PLASMINOGEN ACTIVATION AND INNATE IMMUNE RESPONSES

The vertebrate blood clotting system is evolutionarily a by product of the innate immune system [140, 141], where the blood clotting proteases have diverged from those comprising the complement system.

Here we will take into consideration the increasing body of evidences showing the relationships of fibrin/ogen with the innate immune system and their pathological import.

a. Shift of the Haemostatic Balance in Favour of Clot Formation Within Inflamed Tissues

One of the major consequences of exudation-related vascular leakage is the recruitment of coagulation factors and their activation within inflamed tissues. Activation of coagulation and fibrin deposition as a consequence of inflammation occurs through all the known pathways, is well documented and can be viewed as part of the host defence of the body in an effort to contain the tissue damage, as well as its causative agent and the consequent inflammatory response, to a limited area [142]. As a whole, current understanding of the relationships between inflammation and coagulation points to a shift of the haemostatic balance in favour of clot formation within inflamed tissues [143]. In this context the macrophage plays a pivotal role, together with antigen-presenting cells, neutrophils, endothelial cells and platelets, all provided with pathogen recognition receptors (PRRs). Upon PRRs engagement by DAMPs generated within the inflammation environment, relevant amounts of the main cytokines of the innate immunity (TNF-alpha, IL-1) react with specific receptors, stimulating exposure of tissue factor (TF) on the surface membrane of macrophages, neutrophils, endothelial cells and platelets [144], which initiates the coagulation cascade (Fig. **8**). The shift of the cell surface of the relevant cells toward a coagulation phenotype is not the only effect elicited by the cytokines, but it is only a part of a pleiotropic effect involving cytokines (IL-1, TNF-alpha) and chemokines production, as well as induction of leukocyte adhesion molecules (LAMs) on the surface of endothelial cells [145]. At the same time DAMPs induce complement activation by each one of the complement activation pathways, thereby leading to generation of the chemotactic and anaphylotoxic C3a and C5a and to the membrane attack complex, C5b-9. Many bacteria and antigens recognize and bind circulating mannose-binding lectin, thereby activating the lectin complement pathway. Binding of mannose-binding lectin on the bacterial surface activates MASP2, one of the three mannose-binding lectin-associated serine protease (MASP1, MASP2 and MASP3), which directly converts pro-thrombin to thrombin thus giving origin to a fibrin network [143]. C5a feeds back to promote expression of additional TF. TF-FVIIa triggers the coagulation pathway, leading to activation of FX to FXa (via the tenase complex, involving FVIIIa), and prothrombin (FII) to thrombin (FIIa) (via the pro-thrombinase complex, involving FVa). Interestingly, also the complement membrane attack complex supports thrombin (FIIa) generation (Fig. **8**). Thrombin exhibits a range of pro-inflammatory activities: it induces the release of pro-inflammatory cytokines from endothelial cells, epithelial cells, adipocytes and immune cells [146-148]. Thrombin is chemotactic for monocytes and neutrophils, induces expression of adhesion molecules, and promotes release of chemokines from platelets. Moreover, thrombin directly activates complement factors C3 and C5 [149, 150] and modulates innate immune responses by modifying cytokine secretion and receptor expression by antigen-presenting cells [151]. High doses of thrombin increase endothelial permeability, an important feature of inflammation [152]. Similarly to thrombin, also FXa shows a wide range of pro-inflammatory effects (reviewed in [153]). Most pro-inflammatory activities of thrombin and FXa are mediated by the protease-activated receptors, that play a key role in linking coagulation and inflammation (reviewed in [154]). Moreover, generation of FXa may be facilitated by pathogens, such as viruses [155] without requiring TF. Fibrinogen cleavage by thrombin produces fibrin monomers that will form the primary provisional clot, and two fibrinopeptides (FPA and FPB). FPA is chemotactic and activates leukocytes [156], and FPB exhibits a strong chemotactic activity for monocytes/macrophages [157, 158] (Table **2**). FXIIIa, generated by both FIIa and MASP1, is a trans-glutaminase that cross-links fibrin monomers thus producing a stabile fibrin clot, in parallel with suppression of cytokine release and leukocyte adhesion [143].

b. Fibrin/Ogen, a Danger-associated Molecular Pattern (DAMP), and Fibrin/Ogen Fragments Cause Inflammation

Fibrin formation provides a “provisional matrix” exploited by inflammatory cells and endothelial cells to initiate the process of repair. Microscopic amounts of fibrin are detectable in all tissues which undergo acute inflammation, but an exudate may be defined “fibrinous” only when fibrin deposition is dominant, as in pericarditis, peritonitis, pleuritis, and in chronic arthritis. Layered fibrin cannot persist indefinitely, because macrophages recognize, destroy and digest it, an event which precedes the resolution phase of the inflammation process. The fibrin component of aspecific exudates, as well as a *bona fide* fibrinous exudate is destroyed also by the activation of the fibrinolytic system. Failure of macrophages and of the fibrinolytic system to get rid of fibrin in inflamed sites, preludes evolution toward chronic inflammation.

As fibrin forms, a physiological counter-regulation is set–up, orchestrating its own degradation. Soluble fibrin degradation products (FDP) occur as a result of plasmin-dependent digestion, the process known as fibrinolysis [159]. Although the lysis of fibrinogen is a process that rarely occurs *in vivo*, its study has been exploited to identify possible biological activities of fibrinogen E and

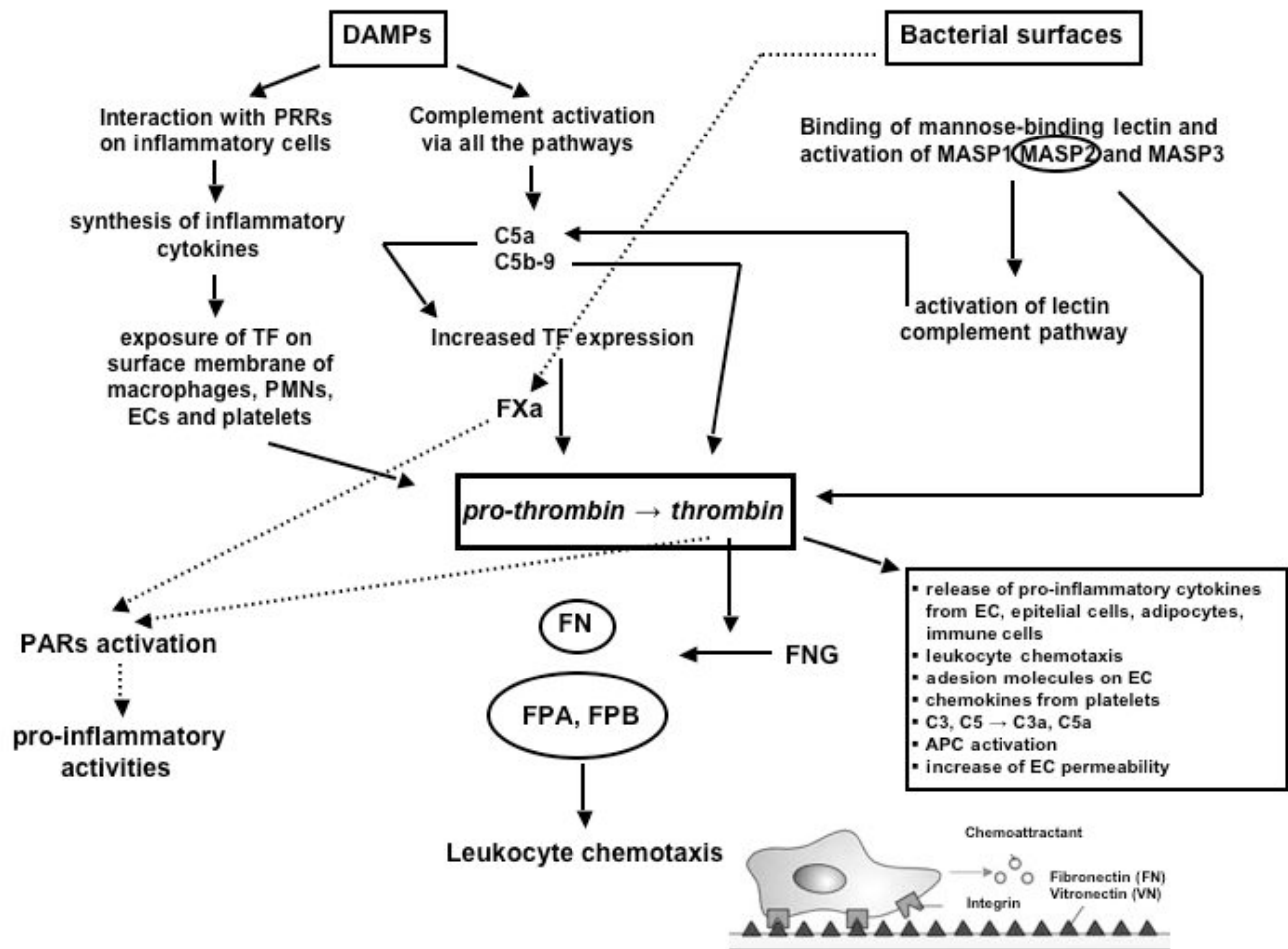

Fig. (8). Activation of thrombin and fibrin generation at the cross-road of inflammation and coagulation/fibrinolysis. Activation of thrombin as a consequence of the inflammatory process occurs through different pathways. The left side of the figure shows DAMPS-dependent thrombin activation. Following DAMPs interaction with PRRs on inflammatory cells, the cytokines of natural immunity (IL-1, TNF-alpha) induce expression of tissue factor (TF) on the surface of macrophages, polymorphonuclear cells (PMNs), endothelial cells (ECs) and platelets, an event that initiates the coagulation cascade, activation of FX to FXa and FXa-dependent activation of pro-thrombin to thrombin. At the same time DAMPs activate complement by the classical, alternative and lectin-dependent pathway, which produce chemotactic and anaphylotoxic C3a and C5a and the membrane attack complex, C5b-9: the complement membrane attack complex supports thrombin (FIIa) generation and C5a feeds back to promote expression of additional TF. Many bacteria and antigens (right part of the figure) recognize and bind circulating mannose-binding lectin, thereby activating the lectin complement pathway. Binding of mannose-binding lectin on the bacterial surface activates MASP2, one of the three mannose-binding lectin-associated serine protease, which directly converts pro-thrombin to thrombin thus giving origin to a fibrin network. TF-FVIIa triggers the coagulation pathway, leading to activation of FX to FXa (via the tenase complex, involving FVIIIa), and prothrombin (FII) to thrombin (FIIa) (via the pro-thrombinase complex, involving FVa). A direct activation of FX to FXa may occur directly on the bacterial surface. Once activated, thrombin activates FNG to FN monomer, releasing fibrinopeptides A and B that express chemotactic activity to leukocytes, and produce the large range of pro-inflammation activities shown in the righ corner box of this figure. Important pro-inflammation activities of thrombin that are not reviewed here are mediated by thrombin activation of protease-activated-receptors (PARs).

D domains, whose structure is shared between fibrin and fibrinogen. After an attack of plasmin, main soluble degradation products are fibrinogen D-fragments consisting of the C-terminal parts, and fibrinogen E-fragments consisting of the N-terminal sections. D fragments are routinely measured as markers for hypercoagulability (as a result of formation and lysis of cross-linked fibrin monomers). The N-terminal aminoacid of the alpha-and beta-chain of E fragments are the same as those of the intact fibrin monomer molecule. These N-terminal sequences represent the only known active sites of E fragments (Fig. **9**). The N-terminus of the alpha-chain interacts with CD18 [160], while the N-terminus of the beta-chain interacts with vascular endothelial (VE)-cadherin. E fragments are rapidly digested into sub-fragments where the N-terminal sequences are truncated and thereby transformed into biologically inactive molecules [161].

Over the last fifteen years, increasing evidence points to involvement of fibrinogen in the inflammatory response.

Critical for generation of fibrin clots, fibrinogen also plays a role in innate immunity and may be viewed as a DAMP (Fig. **9**), since it stimulates monocyte secretion of several cytokines after engagement with toll-like receptor type-4 (TLR4) [162]. Fibrinogen stimulates inflammatory mediators such as IL-1 beta [163], IL-8 [164], macrophage inflammatory proteins, and monocyte chemoattractant protein [165]. Fibrinogen interacts also with integrins on monocytes, macrophages, neutrophils and dendritic cells, and the interaction is strengthened by a more proper integrin conformation induced by uPAR following uPA-uPAR interaction [47-56]. During the inflammatory response, leukocyte-endothelium interactions is a critical event: a specific study has reported *in vivo* that, in addition

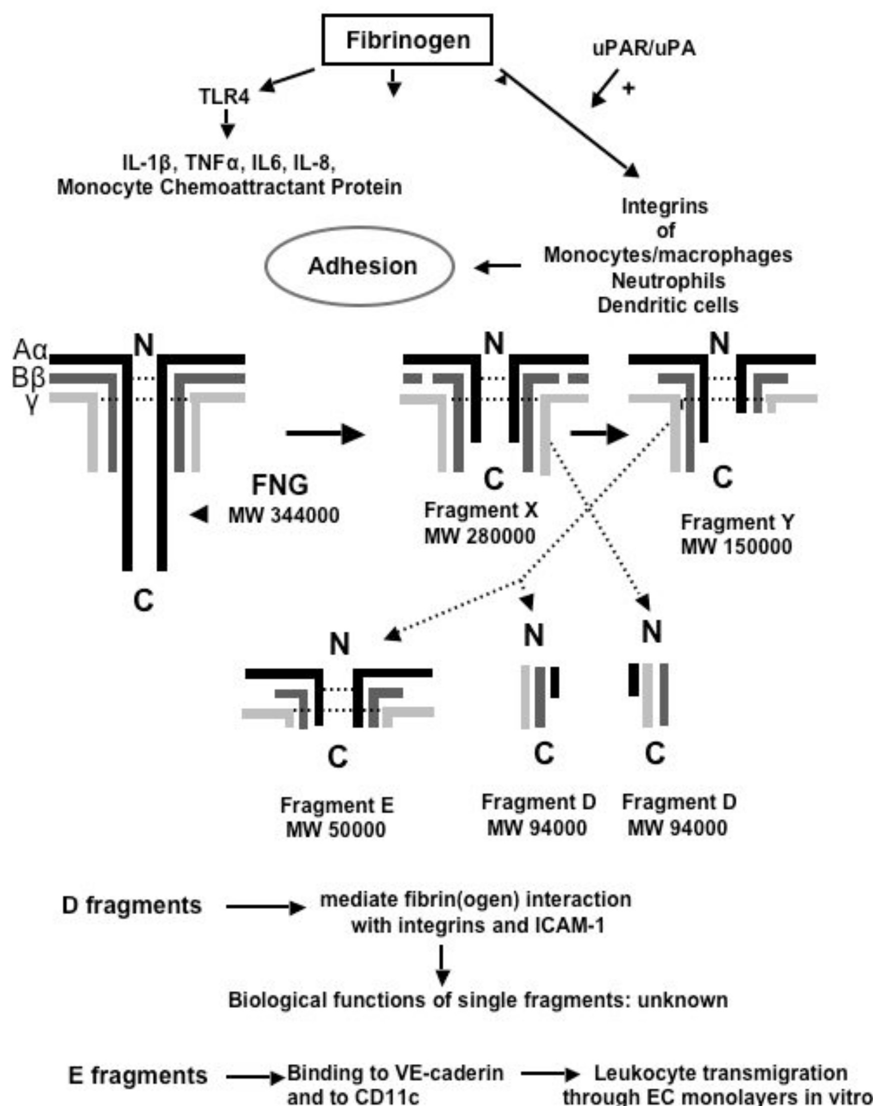

Fig. (9). Pro-inflammatory activities of fibrin(ogen) and of fibrin(ogen) degradation products. The upper part of the figure shows the activity of fibrinogen as a DAMP. Fibrinogen interacts with toll-like receptor type-4 (TLR4), thus triggering secretion of many cytokines of the innate immunity. Fibrinogen interacts also with integrins on monocytes, macrophages, neutrophils and dendritic cells, and the interaction is strengthened by a more proper integrin conformation induced by uPAR following uPA-uPAR interaction, promoting adhesion of innate immunity cells. The lower part of the figure shows a domainal perspective of plasmin-mediated conversion of fibrinogen (FNG) to its fragment D and E, showing the intermediate fragments X and Y. The major plasmin digestion occurs at the C-terminals of the A alpha-chains, the N-terminal of the B beta-chains, and the peptide sequences joining the plasmin-resistant domains D and E. The various structures shown in the figure indicate the polypeptide composition of the various fragments. Although D fragments mediate interaction of fibrinogen with integrins and intercellular-adhesion molecule-1 (ICAM-1), biological functions of single fragments is unknown. On the contrary, isolated single D fragments regulate leukocyte transmigration through endothelial cell monolayers by mediating interaction of leukocyte CD11c to endothelial cell VE-cadherin.

to regulation of IL-6 and monocyte chemoattractant protein, fibrin(ogen) also stimulates macrophage adhesion [166].

Mice deficient in fibrinogen or expressing mutant forms, show a blunted inflammatory response, in particular suppressed macrophage adhesion, cell migration deficit during wound healing, glomerulonephritis and pulmonary fibrosis [167-170]. Additionally, in a model of tumor metastasis, tumor spread via lymphatic and blood vessels is decreased in fibrinogen-deficient mice [171], indicating that fibrinogen and/or its derivatives are involved in mechanisms that regulate transmigration of cells through endothelial cell surfaces.

As described above for products of fibrinogen lysis, also fibrin-derived fragments show pro-inflammatory activities. D-fragments possess binding sites to alphaM/beta2, alphaIIb/beta3, alpha5/beta1, alphav/beta3 or intercellular adhesion molecule-1 (ICAM-1) [172-177]. E-fragments carry a binding site for VE-cadherin [178, 179] as well as for CD11c [160], which induce leukocyte transmigration through endothelial cell monolayers *in vitro* [180] (Fig. **9** and Table **2**).

Overall, these evidences show a still incompletely understood role of both fibrin and fibrin-degradation products. Fibrin formation within the inflammation microenvironment is useful in providing a physical barrier that circumscribes pathogen diffusion, thereby limiting microbial spreading from the damaged tissues. Moreover, fibrin provides a provisional matrix exploited by inflammatory cells to gather into tissue lesions, and a proper molecular track for endothelial cells in the process of vessel formation useful for delivering

Table 2. TYPE-beta-FXIIa Indicates a Proteolysis-derived form of FXII, Also Called Type-beta-activated Hageman Factor (beta-HFa, also Referred to as HFf), Which Derives From a Proteolytic Cleavage of the single chain precursor outside the sequence included between the S-S bridge. This kind of Cleavage Produces Two Distinct Molecules: the NH2-terminal Fragment, Whith so far Unknown Biological Properties, and the COOH-fragment, Containing the Catalytic Site, Showing the Reported Inflammation Properties

Coagulation and Fibrinolytic-derived Pro-inflammatory Effects		
Molecule	**Direct Action**	**Indirect Action**
FXa	➢ Induction of adhesive molecules on monocytes ➢ Induction of pro-inflammatory molecules: IL-1, IL-6, IL-8, MCP-1	Thrombin formation
Thrombin	➢ Chemotactic activity ➢ Induction of P-selectin expression on EC ➢ Induction of secretion of pro-inflammatory molecules: IL-1, IL-6, IL-8, MCP-1	
Fibrinogen	➢ Induction of secretion of: IL-1, IL-8, macrophage inflammatory proteins ➢ Leucocyte adhesion to EC	
Fibirinopeptide B	➢ Chemotactic activity	
uPA /uPAR system	➢ Chemotactic activity ➢ Direct ECM degradation → cell ➢ invasion	
Plasmin	➢ Direct ECM degradation → cell ➢ invasion	
Fibrin degradation products	➢ Vasodilatation (?) ➢ Leucocyte transmigration	Bradykinin interaction (?) → vasodilatation

molecules and cells of tissue repair. At the same time, defective fibrinolysis and extracellular fibrin deposition, such as that occurring in the arthritic joints, causes exacerbation of antigen-induced arthritis [181]. Fibrin could also have a major role in RA pathogenesis as an autoantigen, a property acquired after a deimination process [182]. Therefore, intra-articular fibrin may be considered a factor of perpetuation of joint inflammation [183]. However, the intra-articular injection of uPA directly causes arthritis in mice [184], possibly for the pro-inflammatory activity of fibrin-degradation products. A likely hypothesis is that coagulation and reactive fibrinolysis are simultaneously activated within the RA joint: when fibrinolysis prevails inflammation is short-lived, when coagulation prevails inflammation becomes chronic. An important factor which predisposes to chronic inflammation is the thrombin activated fibrinolysis inhibitor (TAFI), which is abundant in the synovial fluid of RA joints, in contrast to the levels observed in a control population [183]. TAFI is a thrombin-activated carboxipeptidase that cleaves the carboxyl-terminal lysine residues on fibrin, thus down-regulating the cofactor activity for tissue plasminogen activator (tPA) binding to fibrin and the subsequent activation of tPA-dependent fibrinolysis.

Similar considerations hold true for the plasminogen activation system: it is interesting that whereas the plasminogen activation system is on one hand required to prevent extracellular fibrin deposition, and thus be considered as an anti-inflammatory agent, on the other hand it is required to mount innate and adaptive responses, and thus be viewed as a pro-inflammation agent (see also Table **2**). Moreover, uPAR-bound uPA on the surface of synovial cells and of synovial vessel endothelial cells promotes angiogenesis within the synovial pannus and erosion of the underlying bone by activation of the classical plasminogen activation-dependent protease cascade, effects which eventuate into the typical bone erosions that determine impairment of joint function in RA [185, 186].

CONCLUSIONS

We have focused on only a few of the many molecular and cellular pathways that relate coagulation and fibrinolysis to the innate and adaptive immune system in the inflammation process. Many other exist, such as the role of endothelial cells, which normally maintain an hemostatic balance by expressing pro-hemostatic and anti-hemostatic activities, a balance that is unbalanced toward excess pro-coagulation in inflammation. We did not discuss the role of protease activated receptors (PARs), that are recognized as a definite link between coagulation and innate immunity. uPAR itself, which undergoes potentiation of its activities upon uPA binding has recently been proposed as a new type of PAR. Despite major advances in our understanding of the many interactions between the systems, patients still lack therapies for serious illness in which

hypercoagulation, fibrinolysis and inflammation coexist. However, the PA system continues to surprise the scientific community with plenty of unexpected activities in every field of human pathology and we are confident that the increasing body of evidences indicating an intimate integration among fibrinolysis, coagulation and inflammation will provide new directions for the development of many more effective treatment strategies.

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REFERENCES

[1] Mignatti P, Rifkin DB. Biology and biochemistry of proteinases in tumor invasion. Physiol Rev 1993; 73: 161-95.

[2] Choong PF, Nadesapillai AP. Urokinase plasminogen activator system: a multifunctional role in tumor progression and metastasis. Clin Orthop Relat Res 2003: S46-58.

[3] Montuori N, Visconte V, Rossi G, Ragno P. Soluble and cleaved forms of the urokinase-receptor: degradation products or active molecules? Thromb Haemost 2005; 93: 192-8.

[4] Mondino A, Blasi F. uPA and uPAR in fibrinolysis, immunity and pathology. Trends Immunol 2004; 25: 450-5.

[5] Rijneveld AW, Levi M, Florquin S *et al.* Urokinase receptor is necessary for adequate host defense against pneumococcal pneumonia. J Immunol 2002; 168: 3507-11.

[6] Gong Y, Hart E, Shchurin A, Hoover-Plow J. Inflammatory macrophage migration requires MMP-9 activation by plasminogen in mice. J Clin Invest 2008; 118: 3012-24.

[7] Nykjaer A, Moller B, Todd RF, 3rd, *et al.* Urokinase receptor. An activation antigen in human T lymphocytes. J Immunol 1994; 152: 505-16.

[8] Bianchi E, Ferrero E, Fazioli F, *et al.* Integrin-dependent induction of functional urokinase receptors in primary T lymphocytes. J Clin Invest 1996; 98: 1133-41.

[9] Al-Atrash G, Kitson RP, Xue Y, *et al.* uPA and uPAR contribute to NK cell invasion through the extracellular matrix. Anticancer Res 2001; 21: 1697-704.

[10] Al-Atrash G, Shetty S, Idell S, *et al.* IL-2-mediated upregulation of uPA and uPAR in natural killer cells. Biochem Biophys Res Commun 2002; 292: 184-9.

[11] Del Rosso M, Fibbi G, Pucci M, *et al.* The plasminogen activation system in inflammation. Front Biosci 2008; 13: 4667-86.

[12] Gonias SL. Factor XII bridges coagulation and fibrinolysis again. Blood 115: 4979-80.

[13] Colman RW, Wu Y, Liu Y. Mechanisms by which cleaved kininogen inhibits endothelial cell differentiation and signalling. Thromb Haemost 104: 875-85.

[14] Kaplan AP, Joseph K, Silverberg M. Pathways for bradykinin formation and inflammatory disease. J Allergy Clin Immunol 2002; 109: 195-209.

[15] Cochrane CG, Griffin JH. Molecular assembly in the contact phase of the Hageman factor system. Am J Med 1979; 67: 657-64.

[16] Colman RW. Activation of plasminogen by human plasma kallikrein. Biochem Biophys Res Commun 1969; 35: 273-9.

[17] Mandle RJ, Jr., Kaplan AP. Hageman-factor-dependent fibrinolysis: generation of fibrinolytic activity by the interaction of human activated factor XI and plasminogen. Blood 1979; 54: 850-62.

[18] Schousboe I, Feddersen K, Rojkjaer R. Factor XIIa is a kinetically favorable plasminogen activator. Thromb Haemost 1999; 82: 1041-6.

[19] Kluft C, Dooijewaard G, Emeis JJ. Role of the contact system in fibrinolysis. Semin Thromb Hemost 1987; 13: 50-68.

[20] Maas C, Govers-Riemslag JW, Bouma B, *et al.* Misfolded proteins activate factor XII in humans, leading to kallikrein formation without initiating coagulation. J Clin Invest 2008; 118: 3208-18.

[21] Joseph K, Kaplan AP. Formation of bradykinin: a major contributor to the innate inflammatory response. Adv Immunol 2005; 86: 159-208.

[22] Colman RW. Biologic activities of the contact factors *in vivo*--potentiation of hypotension, inflammation, and fibrinolysis, and inhibition of cell adhesion, angiogenesis and thrombosis. Thromb Haemost 1999; 82: 1568-77.

[23] LaRusch GA, Mahdi F, Shariat-Madar Z, *et al.* Factor XII stimulates ERK1/2 and Akt through uPAR, integrins, and the EGFR to initiate angiogenesis. Blood 115: 5111-20.

[24] Skrzydlewska E, Sulkowska M, Koda M, Sulkowski S. Proteolytic-antiproteolytic balance and its regulation in carcinogenesis. World J Gastroenterol 2005; 11: 1251-66.

[25] Collen D, Lijnen HR. Thrombolytic agents. Thromb Haemost 2005; 93: 627-30.

[26] Blasi F, Carmeliet P. uPAR: a versatile signalling orchestrator. Nat Rev Mol Cell Biol 2002; 3: 932-43.

[27] Carmeliet P, Bouche A, De Clercq C, *et al.* Biological effects of disruption of the tissue-type plasminogen activator, urokinase-type plasminogen activator, and plasminogen activator inhibitor-1 genes in mice. Ann N Y Acad Sci 1995; 748: 367-81; discussion 381-2.

[28] Selvarajan S, Lund LR, Takeuchi T, *et al.* A plasma kallikrein-dependent plasminogen cascade required for adipocyte differentiation. Nat Cell Biol 2001; 3: 267-75.

[29] Del Rosso M, Fibbi G, Pucci M, *et al.* Multiple pathways of cell invasion are regulated by multiple families of serine proteases. Clin Exp Metastasis 2002; 19: 193-207.

[30] Herren T, Swaisgood C, Plow EF. Regulation of plasminogen receptors. Front Biosci 2003; 8: d1-8.

[31] Kuiper J, Otter M, Rijken DC, van Berkel TJ. Characterization of the interaction *in vivo* of tissue-type plasminogen activator with liver cells. J Biol Chem 1988; 263: 18220-4.

[32] Fukao H, Ueshima S, Takaishi T, *et al.* Enhancement of tissue-type plasminogen activator (t-PA) activity by purified t-PA receptor expressed in human endothelial cells. Biochim Biophys Acta 1997; 1356: 111-20.

[33] Verrall S, Seeds NW. Characterization of 125I-tissue plasminogen activator binding to cerebellar granule neurons. J Cell Biol 1989; 109: 265-71.

[34] Vassalli JD, Baccino D, Belin D. A cellular binding site for the Mr 55,000 form of the human plasminogen activator, urokinase. J Cell Biol 1985; 100: 86-92.

[35] Wei Y, Waltz DA, Rao N, *et al.* Identification of the urokinase receptor as an adhesion receptor for vitronectin. J Biol Chem 1994; 269: 32380-8.

[36] Hoyer-Hansen G, Behrendt N, Ploug M, *et al.* The intact urokinase receptor is required for efficient vitronectin binding: receptor cleavage prevents ligand interaction. FEBS Lett 1997; 420: 79-85.

[37] Sidenius N, Blasi F. Domain 1 of the urokinase receptor (uPAR) is required for uPAR-mediated cell binding to vitronectin. FEBS Lett 2000; 470: 40-6.

[38] Sidenius N, Andolfo A, Fesce R, Blasi F. Urokinase regulates vitronectin binding by controlling urokinase receptor oligomerization. J Biol Chem 2002; 277: 27982-90.

[39] Kanse SM, Kost C, Wilhelm OG, *et al.* The urokinase receptor is a major vitronectin-binding protein on endothelial cells. Exp Cell Res 1996; 224: 344-53.

[40] Deng G, Curriden SA, Wang S, *et al.* Is plasminogen activator inhibitor-1 the molecular switch that governs urokinase receptor-mediated cell adhesion and release? J Cell Biol 1996; 134: 1563-71.

[41] Asakura S, Yang W, Sottile J, *et al.* Opposing effects of low and high molecular weight kininogens on cell adhesion. J Biochem 1998; 124: 473-84.

[42] Colman RW, Pixley RA, Najamunnisa S, *et al.* Binding of high molecular weight kininogen to human endothelial cells is mediated via a site within domains 2 and 3 of the urokinase receptor. J Clin Invest 1997; 100: 1481-7.

[43] Chavakis T, Kanse SM, Lupu F, *et al.* Different mechanisms define the antiadhesive function of high molecular weight kininogen in integrin- and urokinase receptor-dependent interactions. Blood 2000; 96: 514-22.

[44] Ossowski L, Aguirre-Ghiso JA. Urokinase receptor and integrin partnership: coordination of signaling for cell adhesion, migration and growth. Curr Opin Cell Biol 2000; 12: 613-20.

[45] Xue W, Kindzelskii AL, Todd RF, 3rd, Petty HR. Physical association of complement receptor type 3 and urokinase-type plasminogen activator receptor in neutrophil membranes. J Immunol 1994; 152: 4630-40.

[46] Reinartz J, Schafer B, Batrla R, *et al.* Plasmin abrogates alpha v beta 5-mediated adhesion of a human keratinocyte cell line (HaCaT) to vitronectin. Exp Cell Res 1995; 220: 274-82.

[47] Wei Y, Lukashev M, Simon DI, *et al.* Regulation of integrin function by the urokinase receptor. Science 1996; 273: 1551-5.
[48] Xue W, Mizukami I, Todd RF, 3rd, Petty HR. Urokinase-type plasminogen activator receptors associate with beta1 and beta3 integrins of fibrosarcoma cells: dependence on extracellular matrix components. Cancer Res 1997; 57: 1682-9.
[49] Margheri F, Manetti M, Serrati S, *et al.* Domain 1 of the urokinase-type plasminogen activator receptor is required for its morphologic and functional, beta2 integrin-mediated connection with actin cytoskeleton in human microvascular endothelial cells: failure of association in systemic sclerosis endothelial cells. Arthritis Rheum 2006; 54: 3926-38.
[50] Chaurasia P, Aguirre-Ghiso JA, Liang OD, *et al.* A region in urokinase plasminogen receptor domain III controlling a functional association with alpha5beta1 integrin and tumor growth. J Biol Chem 2006; 281: 14852-63.
[51] Tarui T, Mazar AP, Cines DB, Takada Y. Urokinase-type plasminogen activator receptor (CD87) is a ligand for integrins and mediates cell-cell interaction. J Biol Chem 2001; 276: 3983-90.
[52] Loftus JC, Smith JW, Ginsberg MH. Integrin-mediated cell adhesion: the extracellular face. J Biol Chem 1994; 269: 25235-8.
[53] Zhang F, Tom CC, Kugler MC, *et al.* Distinct ligand binding sites in integrin alpha3beta1 regulate matrix adhesion and cell-cell contact. J Cell Biol 2003; 163: 177-88.
[54] Yebra M, Parry GC, Stromblad S, *et al.* Requirement of receptor-bound urokinase-type plasminogen activator for integrin alphavbeta5-directed cell migration. J Biol Chem 1996; 271: 29393-9.
[55] Yebra M, Goretzki L, Pfeifer M, Mueller BM. Urokinase-type plasminogen activator binding to its receptor stimulates tumor cell migration by enhancing integrin-mediated signal transduction. Exp Cell Res 1999; 250: 231-40.
[56] Tarui T, Andronicos N, Czekay RP, *et al.* Critical role of integrin alpha 5 beta 1 in urokinase (uPA)/urokinase receptor (uPAR, CD87) signaling. J Biol Chem 2003; 278: 29863-72.
[57] Monaghan-Benson E, McKeown-Longo PJ. Urokinase-type plasminogen activator receptor regulates a novel pathway of fibronectin matrix assembly requiring Src-dependent transactivation of epidermal growth factor receptor. J Biol Chem 2006; 281: 9450-9.
[58] Wei Y, Czekay RP, Robillard L, *et al.* Regulation of alpha5beta1 integrin conformation and function by urokinase receptor binding. J Cell Biol 2005; 168: 501-11.
[59] Aguirre Ghiso JA, Kovalski K, Ossowski L. Tumor dormancy induced by downregulation of urokinase receptor in human carcinoma involves integrin and MAPK signaling. J Cell Biol 1999; 147: 89-104.
[60] Aguirre-Ghiso JA, Liu D, Mignatti A, *et al.* Urokinase receptor and fibronectin regulate the ERK(MAPK) to p38(MAPK) activity ratios that determine carcinoma cell proliferation or dormancy *in vivo*. Mol Biol Cell 2001; 12: 863-79.
[61] Aguirre-Ghiso JA, Estrada Y, Liu D, Ossowski L. ERK(MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38(SAPK). Cancer Res 2003; 63: 1684-95.
[62] Montuori N, Carriero MV, Salzano S, *et al.* The cleavage of the urokinase receptor regulates its multiple functions. J Biol Chem 2002; 277: 46932-9.
[63] Cao DJ, Guo YL, Colman RW. Urokinase-type plasminogen activator receptor is involved in mediating the apoptotic effect of cleaved high molecular weight kininogen in human endothelial cells. Circ Res 2004; 94: 1227-34.
[64] Resnati M, Guttinger M, Valcamonica S, *et al.* Proteolytic cleavage of the urokinase receptor substitutes for the agonist-induced chemotactic effect. EMBO J 1996; 15: 1572-82.
[65] Fazioli F, Resnati M, Sidenius N, *et al.* A urokinase-sensitive region of the human urokinase receptor is responsible for its chemotactic activity. EMBO J 1997; 16: 7279-86.
[66] Ragno P. The urokinase receptor: a ligand or a receptor? Story of a sociable molecule. Cell Mol Life Sci 2006; 63: 1028-37.
[67] Resnati M, Pallavicini I, Wang JM, *et al.* The fibrinolytic receptor for urokinase activates the G protein-coupled chemotactic receptor FPRL1/LXA4R. Proc Natl Acad Sci U S A 2002; 99: 1359-64.
[68] de Paulis A, Montuori N, Prevete N, *et al.* Urokinase induces basophil chemotaxis through a urokinase receptor epitope that is an endogenous ligand for formyl peptide receptor-like 1 and -like 2. J Immunol 2004; 173: 5739-48.
[69] Gyetko MR, Todd RF, 3rd, Wilkinson CC, Sitrin RG. The urokinase receptor is required for human monocyte chemotaxis *in vitro*. J Clin Invest 1994; 93: 1380-7.
[70] Furlan F, Orlando S, Laudanna C, *et al.* The soluble D2D3(88-274) fragment of the urokinase receptor inhibits monocyte chemotaxis and integrin-dependent cell adhesion. J Cell Sci 2004; 117: 2909-16.
[71] Liu D, Aguirre Ghiso J, Estrada Y, Ossowski L. EGFR is a transducer of the urokinase receptor initiated signal that is required for *in vivo* growth of a human carcinoma. Cancer Cell 2002; 1: 445-57.
[72] Cunningham O, Andolfo A, Santovito ML, *et al.* Dimerization controls the lipid raft partitioning of uPAR/CD87 and regulates its biological functions. EMBO J 2003; 22: 5994-6003.
[73] Hoyer-Hansen G, Ronne E, Solberg H, *et al.* Urokinase plasminogen activator cleaves its cell surface receptor releasing the ligand-binding domain. J Biol Chem 1992; 267: 18224-9.
[74] Plesner T, Behrendt N, Ploug M. Structure, function and expression on blood and bone marrow cells of the urokinase-type plasminogen activator receptor, uPAR. Stem Cells 1997; 15: 398-408.
[75] Levi M, Schultz M, van der Poll T. Disseminated intravascular coagulation in infectious disease. Semin Thromb Hemost 36: 367-77.
[76] Blasi F. Urokinase and urokinase receptor: a paracrine/autocrine system regulating cell migration and invasiveness. Bioessays 1993; 15: 105-11.
[77] Abraham E, Gyetko MR, Kuhn K, *et al.* Urokinase-type plasminogen activator potentiates lipopolysaccharide-induced neutrophil activation. J Immunol 2003; 170: 5644-51.
[78] Cao D, Mizukami IF, Garni-Wagner BA, *et al.* Human urokinase-type plasminogen activator primes neutrophils for superoxide anion release. Possible roles of complement receptor type 3 and calcium. J Immunol 1995; 154: 1817-29.
[79] May AE, Kanse SM, Lund LR, *et al.* Urokinase receptor (CD87) regulates leukocyte recruitment via beta 2 integrins *in vivo*. J Exp Med 1998; 188: 1029-37.
[80] Gyetko MR, Sitrin RG, Fuller JA, *et al.* Function of the urokinase receptor (CD87) in neutrophil chemotaxis. J Leukoc Biol 1995; 58: 533-8.
[81] Vaday GG, Lider O. Extracellular matrix moieties, cytokines, and enzymes: dynamic effects on immune cell behavior and inflammation. J Leukoc Biol 2000; 67: 149-59.
[82] Gyetko MR, Sud S, Chen GH, *et al.* Urokinase-type plasminogen activator is required for the generation of a type 1 immune response to pulmonary Cryptococcus neoformans infection. J Immunol 2002; 168: 801-9.
[83] Fibbi G, Ziche M, Morbidelli L, *et al.* Interaction of urokinase with specific receptors stimulates mobilization of bovine adrenal capillary endothelial cells. Exp Cell Res 1988; 179: 385-95.
[84] Mignatti P, Rifkin DB. Nonenzymatic interactions between proteinases and the cell surface: novel roles in normal and malignant cell physiology. Adv Cancer Res 2000; 78: 103-57.
[85] Del Rosso M, Fibbi G, Schmitt M, Mignatti P. Proteases and extracellular environment. Thromb Haemost 2005; 93: 190-1.
[86] Del Rosso M, Fibbi G, Matucci Cerinic M. The urokinase-type plasminogen activator system and inflammatory joint diseases. Clin Exp Rheumatol 1999; 17: 485-98.
[87] Hart PH, Vitti GF, Burgess DR, *et al.* Activation of human monocytes by granulocyte-macrophage colony-stimulating factor: increased urokinase-type plasminogen activator activity. Blood 1991; 77: 841-8.
[88] Busso N, Hamilton JA. Extravascular coagulation and the plasminogen activator/plasmin system in rheumatoid arthritis. Arthritis Rheum 2002; 46: 2268-79.
[89] Leizer T, Cebon J, Layton JE, Hamilton JA. Cytokine regulation of colony-stimulating factor production in cultured human synovial fibroblasts: I. Induction of GM-CSF and G-CSF production by interleukin-1 and tumor necrosis factor. Blood 1990; 76: 1989-96.
[90] Gyetko MR, Sud S, Kendall T, *et al.* Urokinase receptor-deficient mice have impaired neutrophil recruitment in response to pulmonary Pseudomonas aeruginosa infection. J Immunol 2000; 165: 1513-9.
[91] Gyetko MR, Sud S, Sonstein J, *et al.* Antigen-driven lymphocyte recruitment to the lung is diminished in the absence of urokinase-type plasminogen activator (uPA) receptor, but is independent of uPA. J Immunol 2001; 167: 5539-42.

[92] Sillaber C, Baghestanian M, Hofbauer R*, et al.* Molecular and functional characterization of the urokinase receptor on human mast cells. J Biol Chem 1997; 272: 7824-32.
[93] Gyetko MR, Chen GH, McDonald RA*, et al.* Urokinase is required for the pulmonary inflammatory response to Cryptococcus neoformans. A murine transgenic model. J Clin Invest 1996; 97: 1818-26.
[94] Estreicher A, Muhlhauser J, Carpentier JL*, et al.* The receptor for urokinase type plasminogen activator polarizes expression of the protease to the leading edge of migrating monocytes and promotes degradation of enzyme inhibitor complexes. J Cell Biol 1990; 111: 783-92.
[95] Kindzelskii AL, Laska ZO, Todd RF, 3rd, Petty HR. Urokinase-type plasminogen activator receptor reversibly dissociates from complement receptor type 3 (alpha M beta 2' CD11b/CD18) during neutrophil polarization. J Immunol 1996; 156: 297-309.
[96] Bohuslav J, Horejsi V, Hansmann C*, et al.* Urokinase plasminogen activator receptor, beta 2-integrins, and Src-kinases within a single receptor complex of human monocytes. J Exp Med 1995; 181: 1381-90.
[97] Lominadze D, Dean WL, Tyagi SC, Roberts AM. Mechanisms of fibrinogen-induced microvascular dysfunction during cardiovascular disease. Acta Physiol (Oxf) 198: 1-13.
[98] Sitrin RG, Todd RF, 3rd, Albrecht E, Gyetko MR. The urokinase receptor (CD87) facilitates CD11b/CD18-mediated adhesion of human monocytes. J Clin Invest 1996; 97: 1942-51.
[99] Simon DI, Rao NK, Xu H*, et al.* Mac-1 (CD11b/CD18) and the urokinase receptor (CD87) form a functional unit on monocytic cells. Blood 1996; 88: 3185-94.
[100] Simon DI, Wei Y, Zhang L*, et al.* Identification of a urokinase receptor-integrin interaction site. Promiscuous regulator of integrin function. J Biol Chem 2000; 275: 10228-34.
[101] Pluskota E, Soloviev DA, Plow EF. Convergence of the adhesive and fibrinolytic systems: recognition of urokinase by integrin alpha Mbeta 2 as well as by the urokinase receptor regulates cell adhesion and migration. Blood 2003; 101: 1582-90.
[102] Pluskota E, Soloviev DA, Bdeir K*, et al.* Integrin alphaMbeta2 orchestrates and accelerates plasminogen activation and fibrinolysis by neutrophils. J Biol Chem 2004; 279: 18063-72.
[103] Roelofs JJ, Rouschop KM, Teske GJ*, et al.* The urokinase plasminogen activator receptor is crucially involved in host defense during acute pyelonephritis. Kidney Int 2006; 70: 1942-7.
[104] Chavakis T, Kanse SM, Pixley RA*, et al.* Regulation of leukocyte recruitment by polypeptides derived from high molecular weight kininogen. FASEB J 2001; 15: 2365-76.
[105] Rijneveld AW, Florquin S, Bresser P*, et al.* Plasminogen activator inhibitor type-1 deficiency does not influence the outcome of murine pneumococcal pneumonia. Blood 2003; 102: 934-9.
[106] Marchesi VT, Florey HW. Electron micrographic observations on the emigration of leucocytes. Q J Exp Physiol Cogn Med Sci 1960; 45: 343-8.
[107] Bianchi E, Bender JR, Blasi F, Pardi R. Through and beyond the wall: late steps in leukocyte transendothelial migration. Immunol Today 1997; 18: 586-91.
[108] Rosenberg GA. Matrix metalloproteinases in brain injury. J Neurotrauma 1995; 12: 833-42.
[109] Muller WA. The role of PECAM-1 (CD31) in leukocyte emigration: studies *in vitro* and *in vivo*. J Leukoc Biol 1995; 57: 523-8.
[110] Smith C. In: Liu JHaD Ed. Adhesion: Its Role in Inflammatory Disease. New York WH Freeman. 1992; 83-115.
[111] Hurley J. Acute Inflammation. Baltimore: Williams & Wilkins; 1972.
[112] Beutler BA. TLRs and innate immunity. Blood 2009; 113: 1399-407.
[113] Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007; 81: 1-5.
[114] Degryse B, de Virgilio M. The nuclear protein HMGB1, a new kind of chemokine? FEBS Lett 2003; 553: 11-7.
[115] Gasque P. Complement: a unique innate immune sensor for danger signals. Mol Immunol 2004; 41: 1089-98.
[116] Gyetko MR, Aizenberg D, Mayo-Bond L. Urokinase-deficient and urokinase receptor-deficient mice have impaired neutrophil antimicrobial activation *in vitro*. J Leukoc Biol 2004; 76: 648-56.
[117] Hovius JW, Bijlsma MF, van der Windt GJ*, et al.* The urokinase receptor (uPAR) facilitates clearance of Borrelia burgdorferi. PLoS Pathog 2009; 5: e1000447.
[118] Wiersinga WJ, Kager LM, Hovius JW*, et al.* Urokinase receptor is necessary for bacterial defense against pneumonia-derived septic melioidosis by facilitating phagocytosis. J Immunol 184: 3079-86.
[119] Pliyev BK, Arefieva TI, Menshikov MY. Urokinase receptor (uPAR) regulates complement receptor 3 (CR3)-mediated neutrophil phagocytosis. Biochem Biophys Res Commun 397: 277-82.
[120] Ross GD. Role of the lectin domain of Mac-1/CR3 (CD11b/CD18) in regulating intercellular adhesion. Immunol Res 2002; 25: 219-27.
[121] Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol 1975; 5: 112-7.
[122] Reyburn H, Mandelboim O, Vales-Gomez M*, et al*. Human NK cells: their ligands, receptors and functions. Immunol Rev 1997; 155: 119-25.
[123] Cerwenka A, Lanier LL. Natural killer cells, viruses and cancer. Nat Rev Immunol 2001; 1: 41-9.
[124] Nykjaer A, Petersen CM, Moller B*, et al.* Identification and characterization of urokinase receptors in natural killer cells and T-cell-derived lymphokine activated killer cells. FEBS Lett 1992; 300: 13-7.
[125] al-Atrash G, Kitson RP, Xue Y, Goldfarb RH. Cooperation of urokinase plasminogen activator and matrix metalloproteinases in NK cell invasion. *In Vivo* 2000; 14: 565-70.
[126] Gellert GC, Goldfarb RH, Kitson RP. Physical association of uPAR with the alphaV integrin on the surface of human NK cells. Biochem Biophys Res Commun 2004; 315: 1025-32.
[127] Gellert GC, Kitson RP, Goldfarb RH. Urokinase-type plasminogen activator receptor crosslinking in an NK cell line increases integrin surface expression by the MAP kinase/ERK 1/2 signaling pathway. J Cell Biochem 2003; 89: 279-88.
[128] Lash GE, Otun HA, Innes BA*, et al.* Interferon-gamma inhibits extravillous trophoblast cell invasion by a mechanism that involves both changes in apoptosis and protease levels. FASEB J 2006; 20: 2512-8.
[129] De Oliveira LG, Lash GE, Murray-Dunning C*, et al.* Role of interleukin 8 in uterine natural killer cell regulation of extravillous trophoblast cell invasion. Placenta 31: 595-601.
[130] Naruse K, Lash GE, Bulmer JN*, et al.* The urokinase plasminogen activator (uPA) system in uterine natural killer cells in the placental bed during early pregnancy. Placenta 2009; 30: 398-404.
[131] Kawahara T, Douglas DN, Lewis J*, et al.* Critical role of natural killer cells in the rejection of human hepatocytes after xenotransplantation into immunodeficient mice. Transpl Int 23: 934-43.
[132] Sonoda KH, Nakamura T, Young HA*, et al.* NKT cell-derived urokinase-type plasminogen activator promotes peripheral tolerance associated with eye. J Immunol 2007; 179: 2215-22.
[133] Van Den Broeck T, Stevenaert F, Taveirne S*, et al.* Ly49E-dependent inhibition of natural killer cells by urokinase plasminogen activator. Blood 2008; 112: 5046-51.
[134] Cohen SD, Israel E, Spiess-Meier B, Wainberg MA. Plasminogen activator is an apparent lymphocyte mitogen. J Immunol 1981; 126: 1415-20.
[135] Gyetko MR, Libre EA, Fuller JA*, et al.* Urokinase is required for T lymphocyte proliferation and activation *in vitro*. J Lab Clin Med 1999; 133: 274-88.
[136] Gyetko MR, Sud S, Chensue SW. Urokinase-deficient mice fail to generate a type 2 immune response following schistosomal antigen challenge. Infect Immun 2004; 72: 461-7.
[137] Stonehouse TJ, Woodhead VE, Herridge PS*, et al.* Molecular characterization of U937-dependent T-cell co-stimulation. Immunology 1999; 96: 35-47.
[138] Woodhead VE, Stonehouse TJ, Binks MH*, et al.* Novel molecular mechanisms of dendritic cell-induced T cell activation. Int Immunol 2000; 12: 1051-61.
[139] Higazi AA, El-Haj M, Melhem A*, et al.* Immunomodulatory effects of plasminogen activators on hepatic fibrogenesis. Clin Exp Immunol 2008; 152: 163-73.
[140] Krem MM, Di Cera E. Evolution of enzyme cascades from embryonic development to blood coagulation. Trends Biochem Sci 2002; 27: 67-74.
[141] Delvaeye M, Conway EM. Coagulation and innate immune responses: can we view them separately? Blood 2009; 114: 2367-74.
[142] Levi M, van der Poll T, Buller HR. Bidirectional relation between inflammation and coagulation. Circulation 2004; 109: 2698-704.

[143] Esmon CT. The impact of the inflammatory response on coagulation. Thromb Res 2004; 114: 321-7.
[144] Opal SM, Esmon CT. Bench-to-bedside review: functional relationships between coagulation and the innate immune response and their respective roles in the pathogenesis of sepsis. Crit Care 2003; 7: 23-38.
[145] Busso N, Chobaz-Peclat V, Hamilton J, *et al.* Essential role of platelet activation via protease activated receptor 4 in tissue factor-initiated inflammation. Arthritis Res Ther 2008; 10: R42.
[146] Drake WT, Lopes NN, Fenton JW, 2nd, Issekutz AC. Thrombin enhancement of interleukin-1 and tumor necrosis factor-alpha induced polymorphonuclear leukocyte migration. Lab Invest 1992; 67: 617-27.
[147] Fujita T, Yamabe H, Shimada M, *et al.* Thrombin enhances the production of monocyte chemoattractant protein-1 and macrophage inflammatory protein-2 in cultured rat glomerular epithelial cells. Nephrol Dial Transplant 2008; 23: 3412-7.
[148] Wadgaonkar R, Somnay K, Garcia JG. Thrombin induced secretion of macrophage migration inhibitory factor (MIF) and its effect on nuclear signaling in endothelium. J Cell Biochem 2008; 105: 1279-88.
[149] Huber-Lang M, Sarma JV, Zetoune FS, *et al.* Generation of C5a in the absence of C3: a new complement activation pathway. Nat Med 2006; 12: 682-7.
[150] Clark A, Weymann A, Hartman E, *et al.* Evidence for non-traditional activation of complement factor C3 during murine liver regeneration. Mol Immunol 2008; 45: 3125-32.
[151] Naldini A, Aarden L, Pucci A, *et al.* Inhibition of interleukin-12 expression by alpha-thrombin in human peripheral blood mononuclear cells: a potential mechanism for modulating Th1/Th2 responses. Br J Pharmacol 2003; 140: 980-6.
[152] Feistritzer C, Riewald M. Endothelial barrier protection by activated protein C through PAR1-dependent sphingosine 1-phosphate receptor-1 crossactivation. Blood 2005; 105: 3178-84.
[153] Krupiczojc MA, Scotton CJ, Chambers RC. Coagulation signalling following tissue injury: focus on the role of factor Xa. Int J Biochem Cell Biol 2008; 40: 1228-37.
[154] Shpacovitch V, Feld M, Hollenberg MD, *et al.* Role of protease-activated receptors in inflammatory responses, innate and adaptive immunity. J Leukoc Biol 2008; 83: 1309-22.
[155] Sutherland MR, Friedman HM, Pryzdial EL. Herpes simplex virus type 1-encoded glycoprotein C enhances coagulation factor VIIa activity on the virus. Thromb Haemost 2004; 92: 947-55.
[156] Richardson DL, Pepper DS, Kay AB. Chemotaxis for human monocytes by fibrinogen-derived peptides. Br J Haematol 1976; 32: 507-13.
[157] Kay AB, Pepper DS, McKenzie R. The identification of fibrinopeptide B as a chemotactic agent derived from human fibrinogen. Br J Haematol 1974; 27: 669-77.
[158] Flick MJ, Du X, Witte DP, *et al.* Leukocyte engagement of fibrin(ogen) via the integrin receptor alphaMbeta2/Mac-1 is critical for host inflammatory response *in vivo*. J Clin Invest 2004; 113: 1596-606.
[159] Gaffney PJ. Fibrin degradation products. A review of structures found *in vitro* and *in vivo*. Ann N Y Acad Sci 2001; 936: 594-610.
[160] Loike JD, Sodeik B, Cao L, *et al.* CD11c/CD18 on neutrophils recognizes a domain at the N terminus of the A alpha chain of fibrinogen. Proc Natl Acad Sci U S A 1991; 88: 1044-8.
[161] Olexa SA, Budzynski AZ, Doolittle RF, *et al.* Structure of fragment E species from human cross-linked fibrin. Biochemistry 1981; 20: 6139-45.
[162] Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. J Immunol 2001; 167: 2887-94.
[163] Perez RL, Roman J. Fibrin enhances the expression of IL-1 beta by human peripheral blood mononuclear cells. Implications in pulmonary inflammation. J Immunol 1995; 154: 1879-87.
[164] Qi J, Kreutzer DL. Fibrin activation of vascular endothelial cells. Induction of IL-8 expression. J Immunol 1995; 155: 867-76.
[165] Harley SL, Sturge J, Powell JT. Regulation by fibrinogen and its products of intercellular adhesion molecule-1 expression in human saphenous vein endothelial cells. Arterioscler Thromb Vasc Biol 2000; 20: 652-8.
[166] Szaba FM, Smiley ST. Roles for thrombin and fibrin(ogen) in cytokine/chemokine production and macrophage adhesion *in vivo*. Blood 2002; 99: 1053-9.
[167] Adams RA, Schachtrup C, Davalos D, *et al.* Fibrinogen signal transduction as a mediator and therapeutic target in inflammation: lessons from multiple sclerosis. Curr Med Chem 2007; 14: 2925-36.
[168] Drew AF, Tucker HL, Liu H, *et al.* Crescentic glomerulonephritis is diminished in fibrinogen-deficient mice. Am J Physiol Renal Physiol 2001; 281: F1157-63.
[169] Drew AF, Liu H, Davidson JM, *et al.* Wound-healing defects in mice lacking fibrinogen. Blood 2001; 97: 3691-8.
[170] Wilberding JA, Ploplis VA, McLennan L, *et al.* Development of pulmonary fibrosis in fibrinogen-deficient mice. Ann N Y Acad Sci 2001; 936: 542-8.
[171] Palumbo JS, Potter JM, Kaplan LS, *et al.* Spontaneous hematogenous and lymphatic metastasis, but not primary tumor growth or angiogenesis, is diminished in fibrinogen-deficient mice. Cancer Res 2002; 62: 6966-72.
[172] Altieri DC, Agbanyo FR, Plescia J, *et al.* A unique recognition site mediates the interaction of fibrinogen with the leukocyte integrin Mac-1 (CD11b/CD18). J Biol Chem 1990; 265: 12119-22.
[173] Altieri DC, Plescia J, Plow EF. The structural motif glycine 190-valine 202 of the fibrinogen gamma chain interacts with CD11b/CD18 integrin (alpha M beta 2, Mac-1) and promotes leukocyte adhesion. J Biol Chem 1993; 268: 1847-53.
[174] Altieri DC, Duperray A, Plescia J, *et al.* Structural recognition of a novel fibrinogen gamma chain sequence (117-133) by intercellular adhesion molecule-1 mediates leukocyte-endothelium interaction. J Biol Chem 1995; 270: 696-9.
[175] Farrell DH, Thiagarajan P, Chung DW, Davie EW. Role of fibrinogen alpha and gamma chain sites in platelet aggregation. Proc Natl Acad Sci U S A 1992; 89: 10729-32.
[176] Suehiro K, Gailit J, Plow EF. Fibrinogen is a ligand for integrin alpha5beta1 on endothelial cells. J Biol Chem 1997; 272: 5360-6.
[177] Yokoyama K, Zhang XP, Medved L, Takada Y. Specific binding of integrin alpha v beta 3 to the fibrinogen gamma and alpha E chain C-terminal domains. Biochemistry 1999; 38: 5872-7.
[178] Bach TL, Barsigian C, Yaen CH, Martinez J. Endothelial cell VE-cadherin functions as a receptor for the beta15-42 sequence of fibrin. J Biol Chem 1998; 273: 30719-28.
[179] Gorlatov S, Medved L. Interaction of fibrin(ogen) with the endothelial cell receptor VE-cadherin: mapping of the receptor-binding site in the NH2-terminal portions of the fibrin beta chains. Biochemistry 2002; 41: 4107-16.
[180] Petzelbauer P, Zacharowski PA, Miyazaki Y, *et al.* The fibrin-derived peptide Bbeta15-42 protects the myocardium against ischemia-reperfusion injury. Nat Med 2005; 11: 298-304.
[181] Busso N, Peclat V, Van Ness K, *et al.* Exacerbation of antigen-induced arthritis in urokinase-deficient mice. J Clin Invest 1998; 102: 41-50.
[182] Masson-Bessiere C, Sebbag M, Girbal-Neuhauser E, *et al.* The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. J Immunol 2001; 166: 4177-84.
[183] So AK, Varisco PA, Kemkes-Matthes B, *et al.* Arthritis is linked to local and systemic activation of coagulation and fibrinolysis pathways. J Thromb Haemost 2003; 1: 2510-5.
[184] Jin T, Tarkowski A, Carmeliet P, Bokarewa M. Urokinase, a constitutive component of the inflamed synovial fluid, induces arthritis. Arthritis Res Ther 2003; 5: R9-R17.
[185] Busso N, Peclat V, So A, Sappino AP. Plasminogen activation in synovial tissues: differences between normal, osteoarthritis, and rheumatoid arthritis joints. Ann Rheum Dis 1997; 56: 550-7.
[186] Guiducci S, Del Rosso A, Cinelli M, *et al.* Rheumatoid synovial fibroblasts constitutively express the fibrinolytic pattern of invasive tumor-like cells. Clin Exp Rheumatol 2005; 23: 364-72.

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