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The plasminogen activation system in inflammation

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1. ABSTRACT

Inflammation is an adaptive response to damage of vascularized tissues, which develops according to a stereotyped sequence governed by the local production of the so-called "chemical mediators of inflammation". Here we review the evidences indicating a role of the plasminogen activation system in the regulation of all the phases of the inflammation process. Plasminogen activation 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. By preventing excess fibrin accumulation in inflamed tissues, the plasminogen activation system also governs the proper evolution of the inflammatory exudates and prevents the possibility of a shift from acute to chronic inflammation.

2. INTRODUCTION

Inflammation may be defined as …."a response to injury of vascularized tissues. Its purpose is to deliver defensive materials (blood cells and fluid) to a site of injury. It is not a state but a process" (1). Such definition is the evolution of a concept which started from inflammation as a disease to inflammation as a useful life-saving reaction. Cornelius Celsus, a writer of the first century, wrote in his "De medicina" a sentence that was destined to become immortal and to travel unmodified throughout the history of medicine: *"Notae vero inflammationis sunt quatuor: rubor et tumor cum calore et dolore"*, which means "Truly, the signs of inflammation are four: redness and swelling with heat and pain". These observations still hold true and have never been improved upon by anybody. All the modern mechanistic explanations involving the role of more or less complicated molecular systems in the inflammatory process have to cope and to conform with the four "cardinal signs of inflammation" codified by Celsus. Here we will review and comment the evidences indicating that the plasminogen activation (PA) system plays a role in the pathogenesis of each one of the Celsus signs, roles that are ancillary in the vascular phases of inflammation (*rubor,*

calor), but that become of critical importance in the cellular phase (*tumor*), leading to the formation of the inflammatory exudates. The sign of *dolor* will be also shown to be intrinsically related to the PA system. Many of the basic mechanisms of the PA system in inflammation have been borrowed by studies on cancer cells, which pioneered all the knowledge on the role of the system in cell invasion and chemotaxis.

3. THE PA SYSTEM, A CELL SURFACE INTEGRATED MULTIMOLECULAR COMPLEX

Over the last twenty years many evidences have been provided, leading to a clear distinction between the functions of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), which is now based on the presence of specific receptors for tPA along the fibrin strands and of uPA on the cell surface (uPAR). Thus, while tPA has been identified as the main plasminogen activator (PA) involved in thrombolysis *in vivo*, uPA and 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 invasive processes required in cancer cells spreading, in angiogenesis and in inflammation (for reviews see 2, 3, 4).

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 metalloproteinases), have been implicated in cell invasion (2), the basic idea being that enzyme release 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 (for a review, 5). This idea has greatly increased over the last decades and now there is no doubt that uPA-mediated plasminogen activation to plasmin (PL) 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 PA 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 integrin receptors (for a review see 6).

3.1. Plasminogen activation by intrinsic (kallikrein, FXIa, FXIIa) and extrinsic activators (uPA and tPA)

Plasminogen (PG) is present in extracellular fluids, including plasma, and localizes within fibrin networks through lysine-binding sites present in the noncatalytic region. Such interaction allows PG activation directly on fibrin strands. Cleavage at the Arg_{561}-Val_{562} bond converts the PG single-chain polypeptide zymogen into plasmin (PL), a broad-spectrum serine-protease consisting of two chains held together by a disulphide bond. PL degrades fibrin and almost all the molecules of ECM either directly or by activation of several matrix metallo-protease (MMP) zymogens (7).

PG is activated to PL by intrinsic and extrinsic activators. Some activators are classified as intrinsic after the evidence that they are generated along with activation of the intrinsic blood coagulation (contact) pathway. 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 coagulation factor XII, prekallikrein, high molecular weight kininogen (HK) and coagulation factor XI (8). PG can interact with the contact system to generate PL. This pathway contributes to about 15% of the total fibrinolytic activity in human plasma (9). Several studies have shown that kallikrein and factors XIIa and XIa can directly activate PG to PL (10-12), as shown in Figure 1A and 1B. The activity of the intrinsic activators is of particular importance in inflammation, as discussed below.

There are two dominant extrinsic plasminogen activators in the body: tPA and uPA. These activators have unique structures that affect the specificity and rate of PL generation. 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 PL itself and by an enlarging series of cathepsins and serine proteases (13). tPA exerts an efficient fibrinolysis by virtue of the presence of tPA-binding sites on fibrin strands, where also PG is localized. tPA-fibrin interaction involves tPA kringle 2, and the catalytic activity of tPA results enhanced upon fibrin binding. tPA-dependent pathway of PG activation is central to the control of fibrinolysis and thrombolysis (14). On the other hand, uPA preferentially interacts with a cell surface receptor (uPAR), thereby activating a cascade of events at the cell surface that include cell movement and invasion, angiogenesis, inflammation (2, 15). 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 PG-deficiency-like overt pro-thrombotic state (16). 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 PG 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 (17).

The PA 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 PG and the extrinsic activators (uPA, tPA) bind specific cellular receptors, creating an amplification loop of plasminogen activation directly on the cell surface,

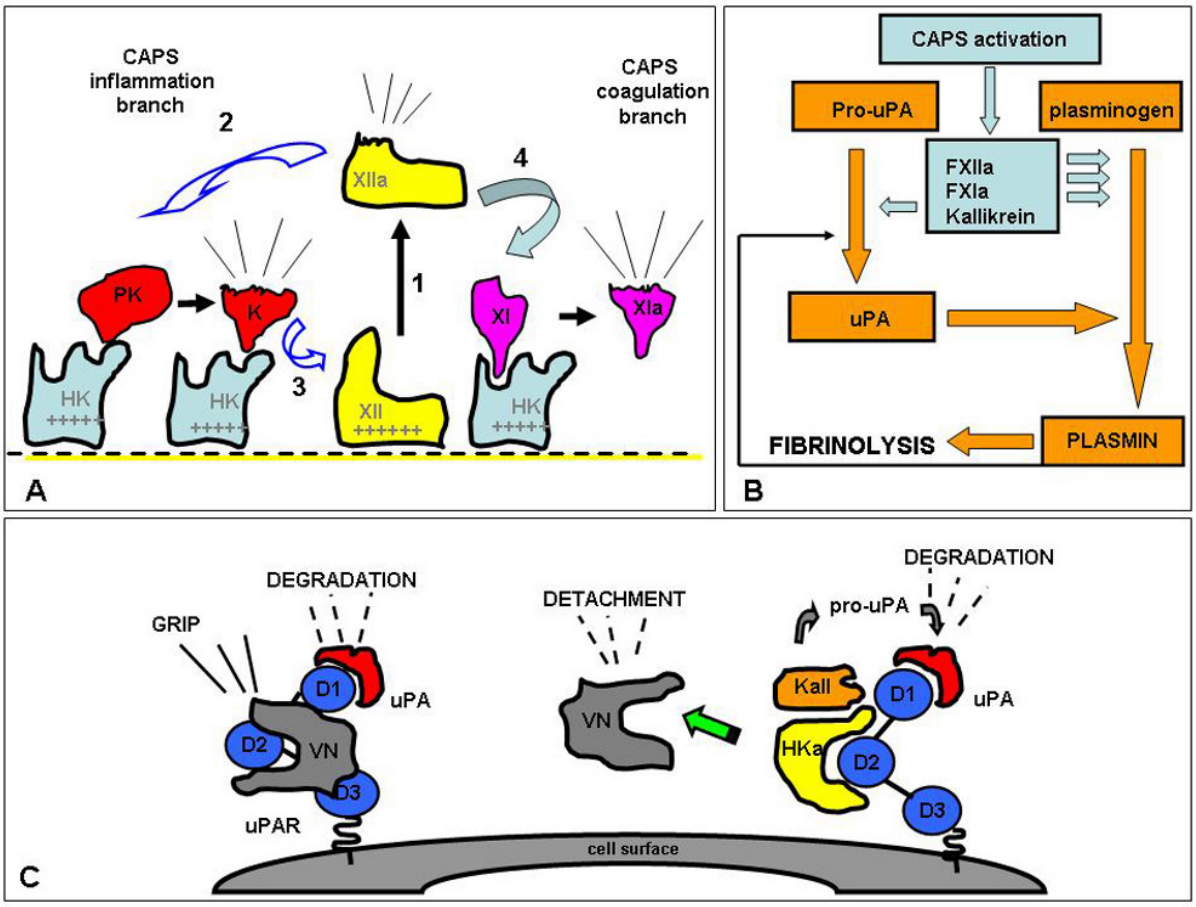

Figure 1. Activation of the "contact-activated plasma system" (CAPS) on negatively-charged surfaces and on the cell surface, and its relationships with fibrinolysis. A: CAPS activation on negative surfaces. The CAPS consists of 4 molecules: coagulation factor XII (FXII or Hageman factor, HF), coagulation factor XI (FXI), plasma prekallikrein (PK), all serine proteinases, and high molecular weight kininogen (HK), which is not a proteinase. Upon exposure of negatively-charged surfaces (dotted line in the Figure), such as bacterial surfaces, basement membranes, ECM proteoglycans, ecc., both FXII and HK bind to the surface by positively-charged aminoacid sequences. FXII undergoes an as yet unexplained auto-activation (step 1 in the Figure), which generates small amounts of activated FXII (FXIIa). HK exhibits two molecular pockets that bind PK and FXI, two molecules which equally share available binding sites on HK. FXIIa, in turn, activates both HK-bound PK to kallikrein (K) (CAPS inflammation branch) (step 2 in the Figure) and HK-bound FXI to FXIa (CAPS coagulation branch) (step 4 in the Figure). PK activation to K triggers an amplification loop which results into a more intense activation of FXII to FXIIa and, as a consequence, a larger generation of K (inflammation) and of FXIa (intrinsic coagulation). The resulting active proteases (K, FXIIa, FXIa) are the so-called "intrinsic activators" of plasminogen (PG), after their property to directly activate PG to PL and pro-uPA to uPA. B: activation of fibrinolysis by intrinsic activators. The CAPS has been originally described as the main plasma system which triggers blood coagulation. Its real importance in blood coagulation was reappraised after the evidence of the preponderant role of the tissue factor (TF)-dependent extrinsic blood coagulation pathways. Clinical and experimental evidences indicate that CAPS activation is of primary importance in inflammation, and fibrinolysis activation by CAPS is the main fibrinolytic pathway within inflamed tissues. C: K(allikrein)-dependent direct activation of fibrinolysis on the cell surface and role of HK in cell detachment. HKa, which lacks the proteolitically released vasodilator peptide bradykinin (BK), binds uPAR in a Zn-dependent manner. HKa competes with vitronectin (VN) for binding to uPAR and dissociates cell-bound VN from uPAR, thereby serving as an anti-adhesive factor. These effects are predominantly attributed to domain 5 of HK (see text and the related Figure 3A). Moreover, HK/HKa binding to cells contributes to the regulation of pericellular PL generation by modulating plasma kallikrein-dependent formation of uPA, a reaction that is dependent on the binding of plasma kallikrein to HK domain 6. Therefore, as shown in the Figure , while the uPAR/VN/uPA complex is able to provide the cell surface with gripping and degradative properties which control cell movement, once VN is displaced from uPAR by HK/HKa, the invasive properties linked to the loss of VN-uPAR interaction and to kallikrein-dependent uPA activation prevail, with an overall pro-degradation attitude of the cell.

able to open a path to invasive cells (18). Three different classes of PG receptors have been identified, able to provide the cell surface with a low-affinity/high-capacity population of pro-invasive molecules (19). Different cell

binding sites for tPA have been described, involved either in tPA clearance in liver cells (20) or in its localization at the surface of the endothelial cell (21) and neuron (22). The cellular receptor for uPA (uPAR) was first described in U937 monocyte-like cells (23) and still is the issue of a number of studies showing its critical role in the regulation of cell movement, invasion and proliferation (2, 15).

Cell surface-associated PG 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 PA system, the protease amplification is mainly the product of the high number of PG/PL binding sites, that usually overcome uPAR/tPA receptors number of about 1-2 orders of magnitude (18).

3.1. The urokinase receptor

The urokinase receptor (uPAR) is a glycosyl-phosptatidyl-inositol (GPI)-anchored protein which, as a consequence of lipid anchor partitioning, is partially located (25%) in cell surface lipid rafts or in specialized forms of rafts, such as caveolae (24) in many types of cells (24, 25). uPAR dimerization favours uPAR recruitment within lipid rafts (26). uPAR is organized in three differently folded homologous domains of about 90 amino acids each, stabilized by intra-molecular disulphide bonds (D1, D2 and D3 from the N-terminus) (27). The recently revealed X-ray structure shows that uPAR binds uPA by directly interacting with D1, in a pocket built by all three domains (28). These structural features suggest the necessity for cooperation of all three uPAR domains for high-affinity binding of uPA or of its amino-terminal fragment (ATF), and allow interactions of the external uPAR molecular surface with other proteins (integrins, vitronectin, EGFR, ecc), a property which will be discussed later (29, 30). uPAR may be anchored to the cell surface either in its native form (D1D2D3) or in a truncated form (D2D3), as a result of a cleavage of the D1-D2 linker region (31). Such a cleavage exposes the chemotactic epitope of uPAR (32). The GPI-anchor cleavage produces the soluble D1D2D3 or D2D3 forms of the receptor (suPAR). All the forms of uPAR so far identified show biological activities (4). Recycling of uPAR between intracellular compartment and the cell surface may be either uPA/PAI-1 dependent via LDL receptor-related protein (33), either constitutive (34) via CD222 (35).

3.2. uPAR ligands: uPA, vitronectin, HKa and a "grip-and-go" model of cell migration

Three extracellular protein ligands involved in ECM degradation and cell adhesion have been identified for u-PAR, namely uPA, vitronectin (VN), high molecular weight kininogen (HK). uPA is secreted as a 54 kDa single chain pro-enzyme, which is activated into the two-chain form by a cleavage at Lys_{158}-Ile_{159}. Within the noncatalytic A chain of uPA an epidermal growth factor (EGF)-like domain (GFD, amino-acids 4-43) and a kringle domain containing three disulphide bonds (KD, amino-acids 47-135) are present. Such modular organization, shared by all the serine proteases, provides the sequence- and stereo-specificity for interaction with binding sites. The A chain or its amino-terminal fragment (ATF, amino-acids 1-135), interact with high affinity with D1 and the D1D2D3 pocket of uPAR (36, 37). The level of glycosylation modulates uPA/uPAR affinity (38). Heparan sulfate (HS) oligomers showing a composition close to the dimeric repeats of heparin exhibit affinity for uPA and confer specificity on uPA/uPAR interaction, indicating a still unexplored role of ECM glycosaminoglycans in modulating uPA-dependent ECM degradation (39). The catalytic C-terminal B chain contains the serine-protease domain, which does not interact with uPAR. uPAR concentrates uPA enzymatic activity on the cell surface, thereby promoting the classic enzyme cascade leading to ECM destruction, a sequence of events that has long been considered the only one required for 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 uPA/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 (18). The identification of vitronectin (VN) as an uPAR ligand provided the first evidence of multiple cell migration-promoting properties of uPAR.

uPAR interacts with the ECM protein VN via the somatomedin B (SMB) domain of VN (40, 41), and with HKa, an activated form of high molecular weight kininogen lacking the vasoactive peptide bradykinin (42). For both molecules binding is mediated by sites located in D2D3 (40, 42), although an efficient binding to VN requires, as in the case of uPA, the full-length uPAR (43). VN binding to uPAR is positively regulated by uPA, likely by inducing the formation of uPAR dimers which, beside being more prone to partition within lipid rafts (26), also show a higher affinity to VN (44). Since VN is a structural ECM molecule, this kind of binding induces on uPAR the gripping properties required for cell migration. PAI-1 also binds VN. Since both binding sites for uPAR and PAI-1 on VN are close to VN SMB domain, PAI-1 may act as an anti-adhesive factor by preventing uPAR/VN interaction (45, 46). 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. Upon contact-activation with negatively-charged surfaces (Figure 1A), the single-chain high molecular weight kininogen (HK) is transformed into the two-chain HKa which undergoes kallikrein-dependent bradykinin release. HKa binds uPAR with high affinity in a zinc-dependent fashion (42) and displaces VN from uPAR, thereby acting as an antiadhesive factor (47) (Figure 1C). Normally, HK shuttles kallikrein, a serine proteinase which is able to activate PG to PL. Therefore, once bound to uPAR, HKa focuses the enzymatic activity of kallikrein on the cell

surface (Figure 1C). This pathway of cell surface-associated PG 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 kininogen.

A large body of evidence indicates that uPA, VN and uPAR itself deliver into the cell proteolysis-independent signals that account for uPAR-dependent cell adhesion, migration and proliferation (15, 48). By its chemical nature, the GPI anchor of uPAR, which deepens into the first layer of the surface membrane phospholipids 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 (4) and are components of a "uPAR transductome" which is assembled on lipid rafts.

3.3. uPAR as a ligand: integrins, GPCR, EGF-R

Convergent biochemical and optical evidences show that beta1, beta2, beta3 and beta5 integrins interact with uPAR on the cell membrane (49-53). Among various integrins, uPAR seems to have the highest affinity to the fibronectin receptors alpha3-beta1 and alpha5-beta1. uPAR domain 3 seems to be relevant in the interaction (54). uPAR interaction with integrins mainly occur in a cis form, but a trans interaction has been described in CHO fibroblasts, which induces cell-cell interaction (55). Regions important for ligand binding by integrins have been mapped to the N-terminal portions of integrin alpha and beta subunits (56). One of these regions corresponds to seven repeats of about 60 aminoacids each that have weak sequence homology to one another. The homologies include FG (phenylalanyl-glycyl) and GAP (glycyl-alanyl-prolyl) sequences. Seven FG-GAP repeats are found in all integrin alpha subunits and many contain putative Ca-binding motifs (57). It was suggested that FG-GAP repeats fold cooperatively into a single domain known as beta-propeller (58), where the term beta refers to the prevalent structure of the aminoacid sequence. In the beta-propeller fold, 4-8 beta-sheets are arranged radially around a pseudo-simmetry axis (59). Each sheet contains four anti-parallel beta-strands that form the legs of a W. These sheets are twisted, like the blades of a propeller, hence the name. 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 (60). 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 (61, 62), alpha5-beta1 signalling (62, 63) and the assembly of a fibronectin matrix (64). uPAR binding induces a conformational change in alpha5-beta1 integrin, which enhances the strength of cell binding to fibronectin in a RGD-independent fashion (65). 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 (66-68). 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 (74). 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) (49). The issue of beta2 integrin-uPAR interaction in neutrophil physiology will be treated in depth in another section of the present review. A recent observation relates integrity of the endothelial cell (EC) uPAR to its possibility to interact with beta2 integrins in order to allow the development of a proper angiogenic program (53). Additionally, only full-size uPAR shows connections with the actin cytoskeleton in EC. Such a connection is mediated by the uPAR-associated alphaM and alphaX subunits of beta2 integrins and results absent in EC of systemic sclerosis, a disease characterized by the absence of angiogenesis even in the presence of a profound tissue hypoxia. EC of systemic sclerosis express on their surface a cleaved uPAR (D2D3), which is unable to interact with integrins and to transduce uPAR-dependent integrin signalling. In human umbilical vein endothelial cells, uPAR forms a signalling complex containing alphav-beta3 or alpha5-beta1, caveolin, and src kinase Yes (69). uPAR has been shown to associate with beta1 and beta2 integrins of fibrosarcoma cells (70).

uPAR can act as a migration, proliferation and adhesion factor by shifting its association with its transmembrane partners. 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 (15).

fMLP is a pyogenic bacteria-specific formylated peptide that stimulates chemotaxis of polymorphonuclear (PMN) granulocytes by activating seven transmembrane domain G-protein-coupled receptors (4). 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 (4). 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) (32, 71, 72). The cleaved suPAR, as well as peptides containing the sequence SRSRY, bind FPRL1 in monocytes, thereby stimulating their migration (73). Full-length GPI-anchored uPAR interacts with FPR through the specific SRSRY sequence (74) and can be considered as an endogenous ligand for fMLP receptors (73,75), whose expression is required for fMLP-directed migration of monocytes and epithelial cells (74, 76).

Another uPAR receptor is the EGFR. This receptor coimmunoprecipitates with uPAR and is activated by high levels of uPAR, such activation is alpha5-beta1 integrin-dependent and determines cell proliferation in vivo (77).

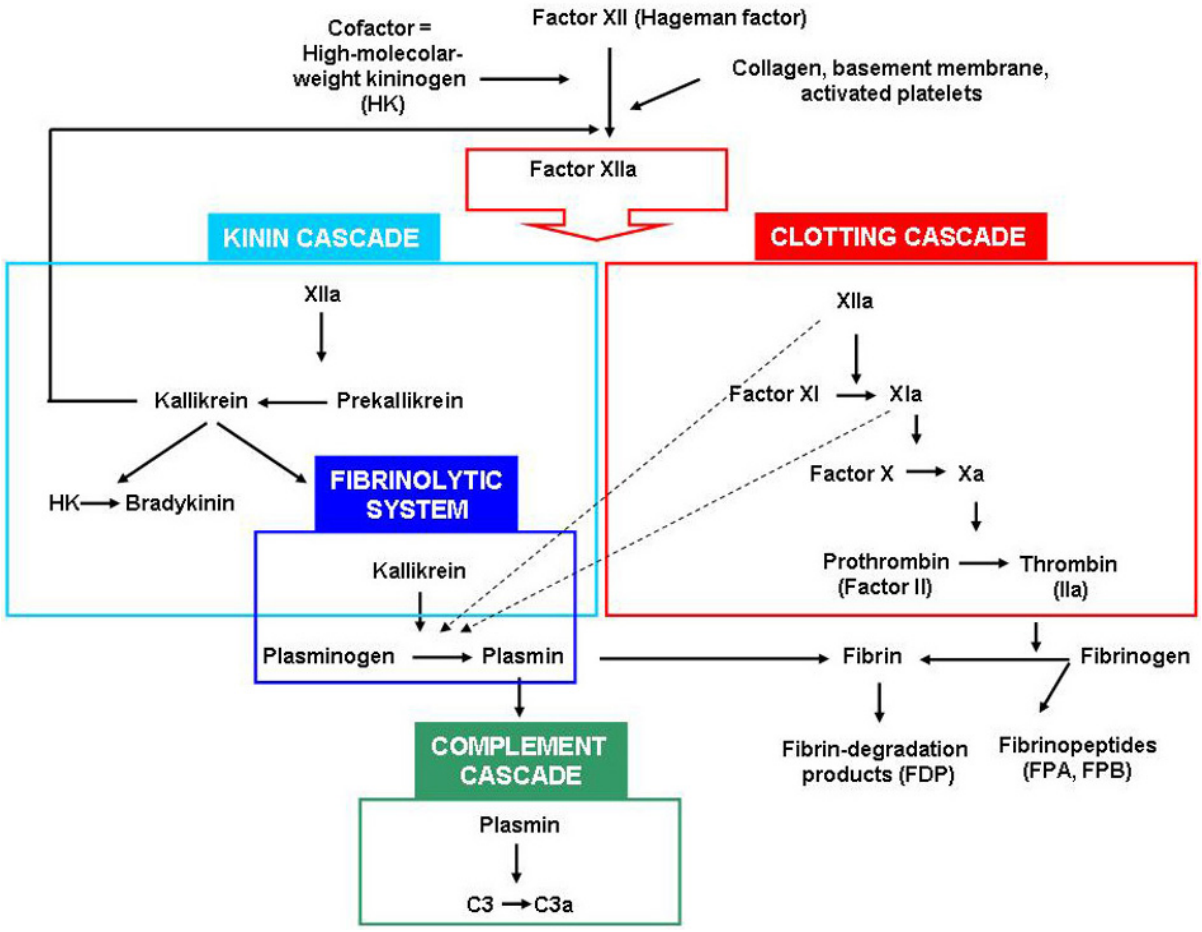

Figure 2. Role of CAPS in kinin generation, clotting, fibrinolysis and complement activation. The Figure shows the complex multiple relationships which regulate the interplay among the molecules activated by CAPS activation. It is noteworthy that not only the kinin and clotting cascades undergo direct activation, as shown in Figure 1A, but also fibrinolysis (refer to intrinsic activators shown in Figure 1B) and complement cascade. Coagulation and complement activation amplify the pro-inflammation activities of the system (fibrino-peptide B, FPB, and formation of C3b, C3a, C5a) with an overall expansion of the formation of powerfull inflammation mediators.

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 (26, 78). 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.

4. RUBOR AND CALOR (REDNESS, HEAT): RELATIONSHIPS BETWEEN PA SYSTEM AND VASODILATATION

Redness and heat of inflamed tissues are the consequence of "too much blood" contained within arterioles, venules and capillaries in the site of damage. Dilatation is the cumulative effect of vasoactive mediators, mainly of histamine. As we will discuss later when considering the cardinal sign of swelling (*tumor*), vessel permeability of an inflamed site is increased, which results into the leakage of plasma proteins in the extra-vascular compartment. Among the plasma proteins, the intrinsic activators of PG (kallikrein, factors XIIa and XIa, which directly activate PG to PL) (10-12), are endowed with properties related with vasodilatation. Tissues undergoing an inflammatory process, as well as many foreign substances, contain negatively-charged surfaces able to recruit and activate the molecules of the so-called "contact-activated plasma system" (CAPS), as shown in Figure 1. The CAPS-related intrinsic PG activators generate PL, which is able to activate the classic complement pathway (Figure 2) (79) or to act directly on C3 or C5 (80, 81), thus generating C3a and C5a. C3a and C5a are members of the family of anaphylatoxins (C3a, C4a, C5a), so called for their property to cause mast cell degranulation upon interaction with specific receptors located on the mast cell surface, which results in histamine-dependent vasodilatation and increase of venules permeability. Such

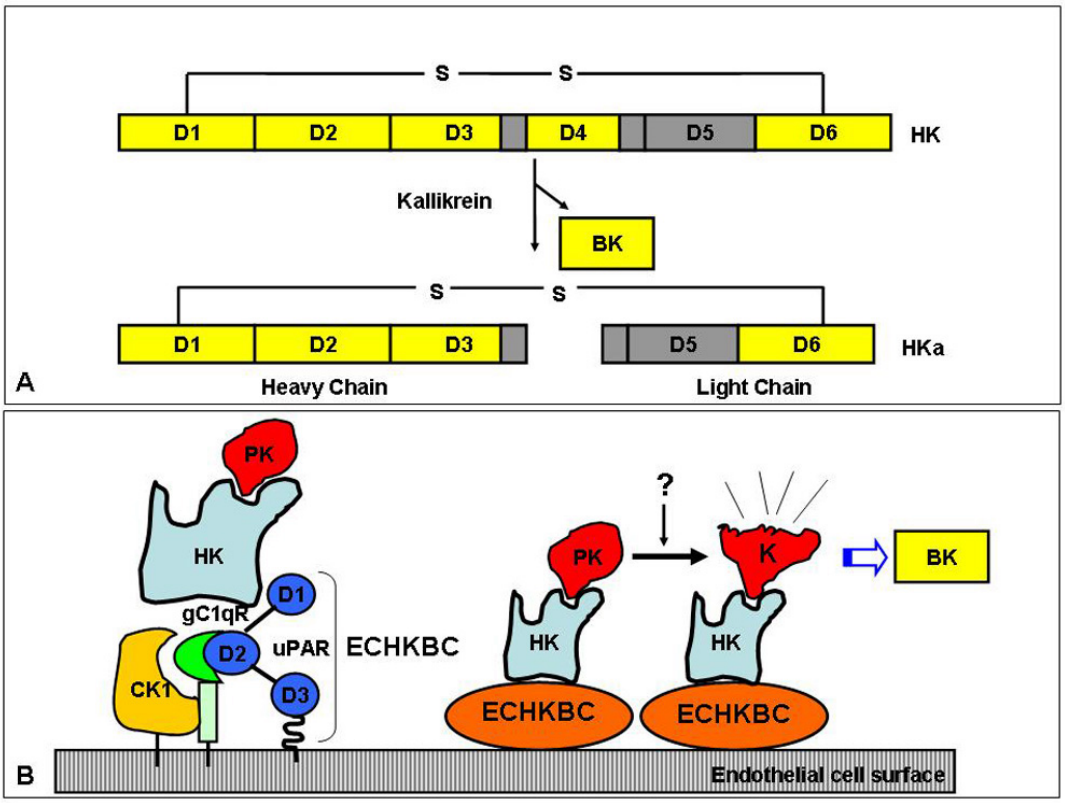

Figure 3. Domain organization of HK and BK generation. A: domain organization of HK. The BK sequence is enframed within domain 4 of HK. Domain 5 of HK interacts with uPAR D2 and D3, displacing VN, as shown in Figure 1C. Domain 6 of HK binds (pre)kallikrein, as shown in Figure 1C. B: generation of BK on the endothelial cell surface. The Figure shows an alternative way of BK generation directly on the surface of endothelial cells. HK/HKa interacts with uPAR, globular C1q receptor (gC1qR) and cytokeratin-1 (CK1), three molecules shown to co-localize on the surface of endothelial cells. The uPAR-CK1-gC1qR complex (here referred to as "endothelial cell high molecular weight kininogen binding complex", ECHKBC), is responsible for HK and prekallikrein (PK) binding to endothelial cells and subsequent release of BK. FXIIa, heat shock protein 90 or prolylcarboxypeptidase are putative activators of PK to K.

pathway of complement activation is not physiological and seems to occur only when PL generation overwhelms its specific and aspecific inhibitors, a situation which easily occurs within inflamed tissues, as a consequence of PG activation to PL by intrinsic activators. There is evidence that anaphylotoxins can also increase vascular permeability directly (82).

Generation of bradykinin (BK) through interaction of uPAR with CAPS molecules provides another functional link between the plasminogen activation system and vasodilatation. Understanding of this topic deserves a more in depth discussion of the properties of high molecular weight kininogen (HK). 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 BK (Figure 3). After BK release by proteolysis, as shown in Figure s 2 and 3, the cleaved HK (HKa) contains a heavy chain and a light chain that remain connected by a disulfide bond (Figure 3). Endothelial cells may bind HK, which becomes a substrate for kallikrein-dependent BK generation from HK D4. Several endothelial cell membrane proteins that interact with HK/HKa have been identified, including uPAR (42), globular C1q receptor (gC1qR) and cytokeratin-1 (CK1) (83, 84, 85), three molecules shown to co-localize on the surface of endothelial cells (86). The uPAR-CK1-gC1qR complex is responsible for HK and prekallikrein binding to endothelial cells and subsequent release of BK (87). BK is a potent vasodilator directly relaxing smooth muscle by releasing PGI2, increases capillary permeability by opening tight junctions between endothelial cells and directly stimulates nerve endings causing pain (88).

5. TUMOR (SWELLING): THE PA SYSTEM IN EXUDATION (FLUID AND PROTEIN LEAKAGE, CHEMOTAXIS AND RECRUITMENT OF WHITE BLOOD CELLS)

Exudate, a well programmed mixture of fluid, proteins and cells, is the unique product of the inflammatory response. Acute inflammation disrupts the equilibrium of fluid and protein exchange in the microcirculation. While vasodilatation (mainly of

arterioles) alters the hydrostatic force that drive fluid out of the vessels, a process where the plasminogen activation system plays a part in the generation of the relevant mediators (BK formation, anaphylatoxin generation and the subsequent histamine release from mastocytes), protein leakage out of dilated venules requires leaks of the endothelial wall. All the plasminogen activation-related mediators previously discussed are endowed with properties related to histamine, which include endothelial contraction leading to venular leakage. While these mediators account for the "immediate vascular leakage", cytokines (IL-1, TNFalpha, IFNgamma) are responsible for the sustained "delayed vascular leakage". Recent results indicate a pathogenic role for the plasminogen activation system even in the delayed vascular leakage. It was known that endogenously produced uPA amplifies TNFalpha secretion by mononuclear phagocytes (89) and potentiates lipopolysaccharide-induced neutrophil activation, which is coupled with cytokine release (90). The monocyte provides the "second wave" of cells within inflamed tissue. The monocyte is known to display uPAR, the beta2 integrin Mac-1 (CD11a/CD18) and gC1qR. HK/HKa contains 2 of its 6 domains involved with cell binding. Both D3 and D5 are needed for binding to Mac-1 (91), but only D5, which is highly exposed in HKa, is needed for binding to uPAR. The simultaneous stimulation of all three receptors by HKa is capable of releasing both inflammatory cytokines TNFalpha, IL-1beta and IL-6 (all responsible for the delayed vascular leakage) and chemokines IL-8 and MCP-1 from human blood mononuclear cells, a series of events that involves p38, JNK and NFkB activation (92).

The purpose of inflammation is to convey fluid and cells to a site of injury. The fluid, with its protein content, is delivered within seconds (immediate vascular leakage) or few hours (delayed vascular leakage). The very first entrance of cells within a site of damage requires at least minutes and hours, since cells cannot just be leaked from the vascular system. 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.

Mononuclear cells represent a reference standard for uPAR itself, since the very first demonstration of a *bona fide* receptor for uPA on U937 monocyte-like cells (23). The first evidence of a possible role of uPAR in leukocyte migration was obtained in the same cells following 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 (4): such stimulation resulted in clustering of uPAR at the leading edge of migrating cells (93). 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) (49). Following migration-related cell polarization, uPAR and Mac-1 dissociates, since uPAR accumulates at lamellipodia and MAC-1 in uropods (94). uPAR and beta2 integrins have been shown associated with Src signalling molecules in large receptor complexes in mononuclear cells (95). 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 the recruitment of PMN and monocytes at sites of inflammation. Fibrinogen, which is present within inflamed tissues as a result of vessel leakage, is another preferential substrate for Mac-1, determining an adhesion-migration substrate for the invading 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 VN. uPAR association with Mac-1 enhances the adhesive function of Mac-1, whereas uPAR occupancy by uPA weakens cell adhesion (96). Transforming growth factor-beta1 and vitamin D3 up-regulate uPAR and Mac-1 expression on human monocytes, enhancing uPAR affinity for VN, 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/PL cascade. The other uPAR ligand, uPA, inhibits Mac-1-dependent fibrinogen binding and degradation (97). This is an example of a finely-tuned molecular system that may alternatively regulate the gripping and the degradation properties of the same cell (Figure 4), in a series of alternating cycles of adhesion and invasion 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 (98).

The ligand binding I-domain of Mac-1 alphaM chain directly interacts with uPA, a binding that impairs integrin functions (96, 99). 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 (99, 100). The critical role of uPAR in regulating

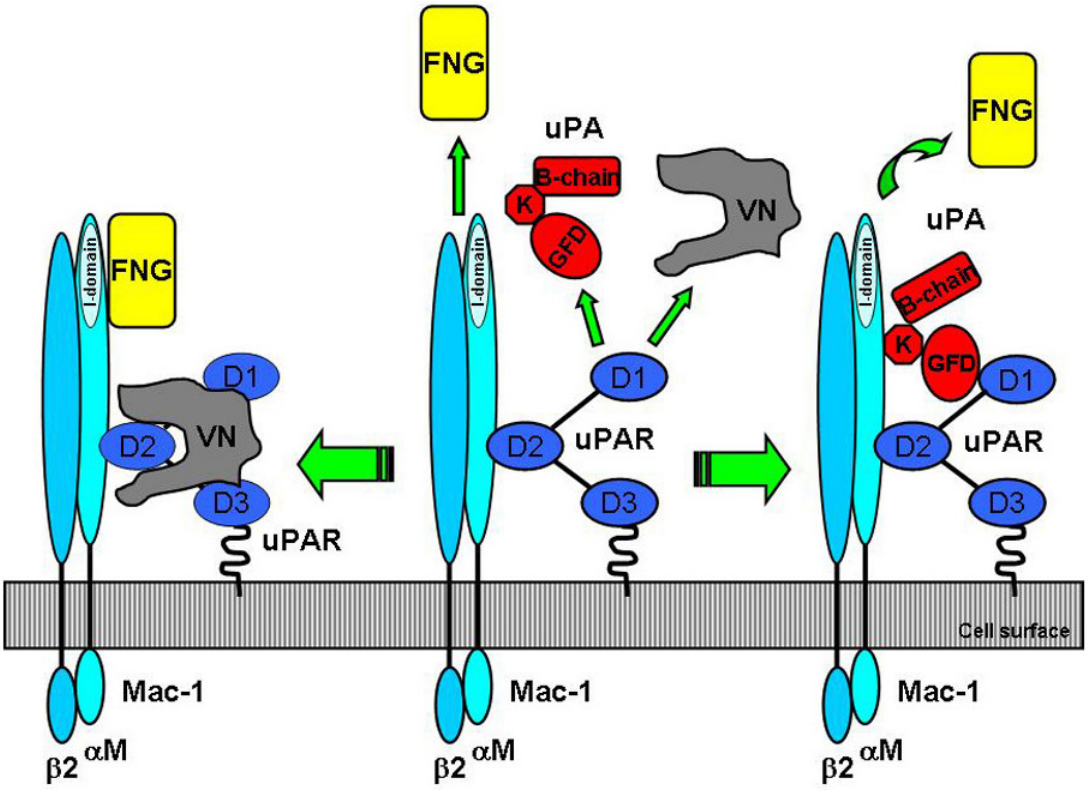

Figure 4. uPAR interactions with beta2 integrin (Mac-1). uPAR is shown in its domain organization (D1-D2-D3). uPA is shown in the modular structure (growth factor domaun, GFD; kringle domain, K; serine protease domain, B chain). uPAR can bind uPA and vitronectin (VN). The beta2 integrin Mac-1 binds fibrinogen (FNG). uPAR interacts with the alphaM chain of Mac-1. Interaction of VN with uPAR (left side of the Figure) promotes binding of Mac-1 to FNG. This first type of interaction (uPAR/VN/Mac-1/FNG) promotes leukocyte adhesion to ECM and to fibrin(ogen) provisional matrix. Interaction of uPAR D1 with uPA (right side of the Figure) weakens interaction of Mac-1 with FNG, probably because of uPA interaction with the ligand binding site (I-domain) of Mac-1 (modified from ref. 4). This second type of interaction (uPAR/Mac-1/uPA), favours leukocyte detachment and invasion.

neutrophil recruitment in inflamed sites has been shown in vivo (101). 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 (102). 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 (103).

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 (47, 104). The described anti-inflammatory properties of HK are independent of uPAR, and they identify HK as a controller of cell adhesion balance by preventing excessive leukocyte recruitment and hyperinflammatory responses.

To reach an inflamed site, leukocytes obey to the chemical call of chemotactic factors. As we have discussed above, no call 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 PA itself. The very first observation of a chemotactic activity of the non-proteolytic A chain of uPA on uPAR-expressing cells goes back to 1988 (105), when it was shown that exogenous uPA could stimulate chemotaxis of human endothelial cell in the Boyden chamber system, 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 (106, 107). The production of uPA in inflamed tissues has been observed mainly in joint inflammatory pathologies and has been extensively reviewed elsewhere (108). 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 inflammation monocyte/macrophage (109). Under appropriate stimuli, such as LPS, monocytes can

themselves produce GM-CSF and G-CSF, initiating an autocrine loop which leads to enhanced production of uPA (110). The same stimuli also induce monocytes to secrete interleukin 1 (IL-1) and tumor necrosis factor alpha (TNFalpha which, in turn, induce production of uPA, GM-CSF and G-CSF from synoviocytes (111) and chondrocytes (110). 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 (75). 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 (103, 112, 113). Chemotaxis of inflammatory cells stimulated by uPA in vitro and in vivo requires binding to uPAR (32, 114, 115) and the presence of a transmembrane adapter able to transduce the chemotactic stimulus (32, 72).

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.

6. DOLOR (PAIN): THE PA SYSTEM AND GENERATION OF PAIN MEDIATORS (KININS)

The pain referred to an inflamed tissue arises when specialized nerve-fiber endings are stimulated by mediators, mainly BK which cause a burning dysaesthesia. Standard "blister test" for the study of pain mediators in humans shows that BK is about 50 times more potent that vasoactive amines (histamine and serotonine) in eliciting pain (116). It is possible that increased tissue pressure due to local swelling may enhance pain, but there is not clear evidence of correlation between the degree of swelling and the degree of pain; non-inflammatory edema is painless. Prostaglandins sensitize the nerve endings to the effect of BK and other algogens (117). Once again, we refer to BK generation from CAPS, involving either uPAR-independent and uPAR-dependent pathways, as shown in Figures 1-3. A powerful mechanism of control, which prevents excessive BK activity, is its enzymatic degradation by specific enzymes (Figure 5). The enzymes that destroy the 9 amino acid peptide BK (arg-pro-pro-gly-phe-ser-pro-phe-arg) consist of kininases I and II. Kininase I, also known as plasma-carboxypeptidase N, removes the C-terminal arg from BK to yield des-arg^9 BK. Kininase II is identical to angiotensin-converting enzyme (ACE). It is a dipeptidase that cleaves the C-terminal phe-arg from BK to yield a heptapeptide, which is cleaved once again to remove ser-pro and to leave the pentapeptide arg-pro-pro-gly-phe (118). Two BK receptor subtypes have been recognized. The B_2 receptor is constitutively expressed in various tissues, including endothelium (119, 120), and is responsible for the majority of BK effects in vitro and in vivo, including the regulation of local and systemic hemodinamics (121). The B_1 receptor has higher affinity for some BK metabolites (des-arg^9 BK) and is induced in tissue damage and inflammation (122). Stimuli for B1 receptor transcription include IL-1 and TNFalpha (123, 124). The binding of BK to receptors activates NO-cGMP and prostacyclin-cAMP signalling pathways (125, 126).

7. THE PA SYSTEM AND THE EVOLUTION OF THE INFLAMMATORY FIBRINOUS EXUDATES

The inflammatory exudate is not a standardized mixture: leukocytes, serum proteins and, to some extent, red blood cells, mix together in various proportions, usually depending on the inflamed tissue as well as on the inflammatory agent. 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 (127). 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 (128). Fibrin formation also 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. This is the case when a thick fibrin deposit coats the heart (pericarditis), the peritoneum (fibrinous peritonitis) or the pleura (fibrinous pleuritis). 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 clear inflamed sites of fibrin, preludes evolution toward chronic inflammation.

Among the rheumatic diseases, "rice bodies" composed by fibrin and collagen fragments in the synovial fluid are patognomonic of chronic synovial inflammation in

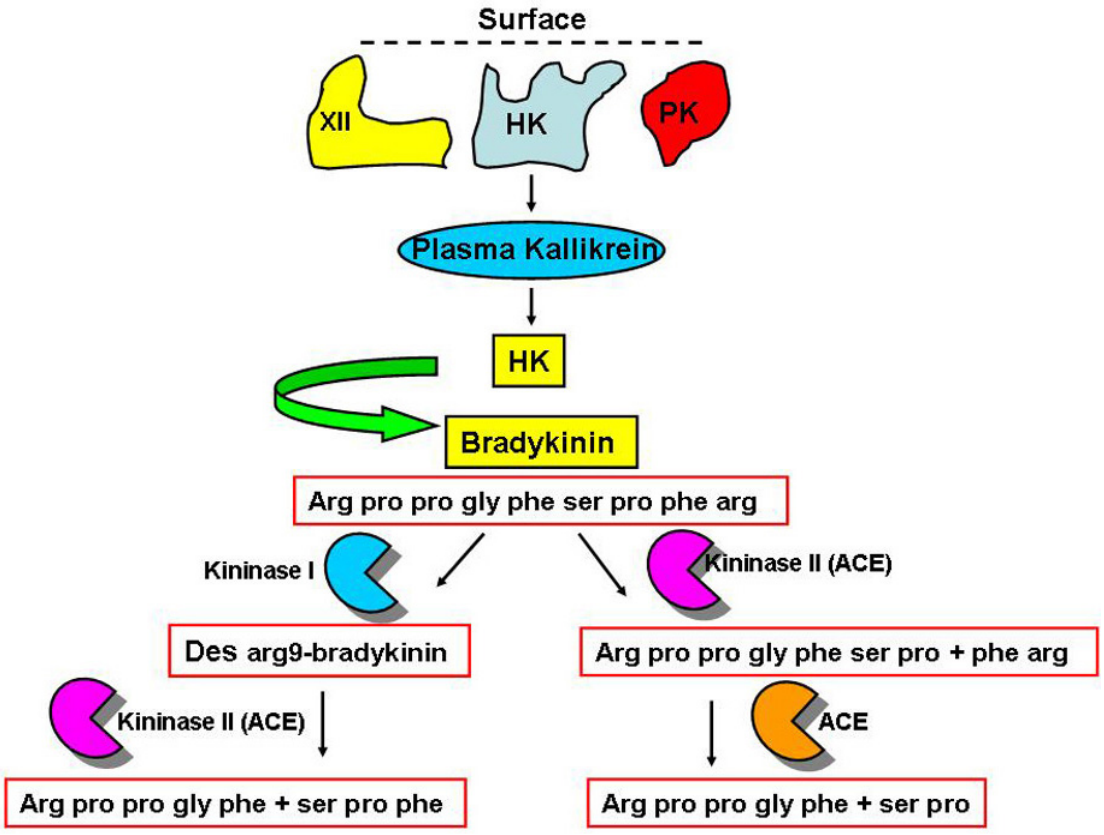

Figure 5. Pathways for formation and degradation of BK. BK formation is determined by the interplay of FXII, HK and PK, as shown in Figure s 1-3 and in the upper part of the present Figure . BK is a peptide which consists of 9 aminoacids, rnumbered from NH2-arg^{1} to COOH-arg^{9}. BK is degraded through two different pathways: the one shown on the left, which involves kininase 1 able to cleave the C-terminal arg^{9} to yield des arg^{9}-BK, and kininase 2 (angiotensin converting enzyme, ACE), which cleaves the last three aminoacids (ser pro phe) of des arg^{9}-BK; the pathway shown on the right, which involves two subsequent steps of kininase II (ACE): the first step cleaves the last two C-terminal aminoacids (phe arg), while the second step produces two peptides (arg pro pro gly phe, and ser pro). Kininase I is the same enzyme that cleaves the C-terminal arg from the complement anaphylatoxins C3a and C5a. BK stimulates constitutively produced B2 receptors, whereas des-arg^{9}-BK stimulates most efficiently B1 receptors, which are induced as a result of inflammation. All the other BK degradation products do not show appreciable biological activities.

Rheumatoid Arthritis (RA). Persistence of fibrin and of fibrin degradation products (FDP) within the inflamed joint is deleterious because of their pro-inflammatory properties (129). Fibrinogen activation derivatives, such as fibrinopeptide B, induce chemotaxis at sites of inflammation (130). Direct roles of fibrin(ogen) and its degradation product, D-dimer, in inflammatory gene expression have been demonstrated. Fibrin and D-dimer enhance monocytic expression of pro-inflammatory cytokines IL-1□ and IL-6 (131, 132), and endothelial production of IL-8 (133). Fibrin(ogen) induces intercellular adhesion molecule-1 and chemokine expression in human synovial fibroblasts, indicating that synovial fibrin(ogen) promotes recruitment of leukocytes within the arthritic joint (134). Fibrin could also have a major role in RA pathogenesis as an autoantigen, a property acquired after a deimination process (135). Therefore, intra-articular fibrin may be considered a factor of perpetuation of joint inflammation (136). 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 (136). 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 (Figure 6). However, an active uPA-dependent fibrinolysis within the synovial fluid has been shown to induce arthritis, an effect due to the pro-inflammatory properties of FDP (137). Moreover, uPAR-bound uPA on the surface of synovial cells and of synovial vessel endothelial cells promote angiogenesis within the synovial pannus and erosion of the underlying bone by activation of the classical plasminogen activation-dependent protease cascade, events which eventuate into the typical bone erosions that determine impairment of joint function in RA (138, 139).

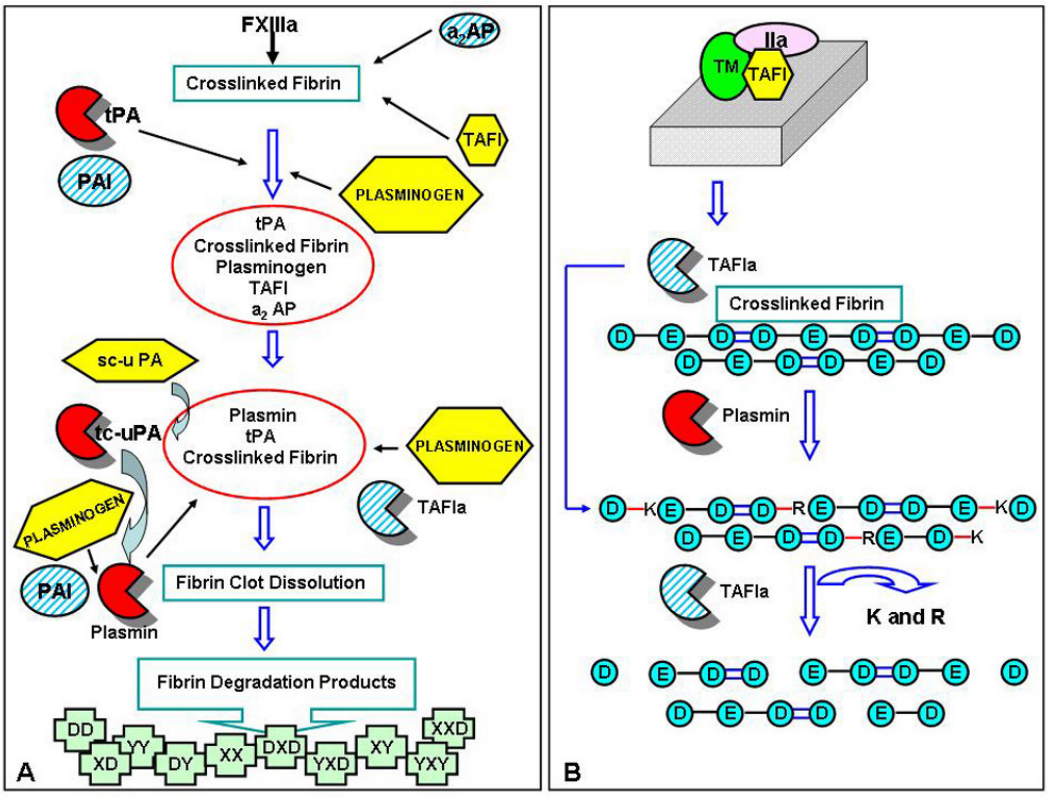

Figure 6. Fibrin formation and dissolution and TAFI activity. A: scheme of the dynamic interaction between the proteins and inhibitors of fibrinolysis. Once formed, fibrin becomes the substrate of two opposite activities, cross-linking and further fibrin deposition and clot dissolution. The key proteins involved are plasminogen, plasminogen activators (tissue type, tPA; single-chain urokinase-type, scuPA; and two-chain urokinase type, or uPA), plasminogen activator inhibitor-1 (PAI-1), alpha$_2$-antiplasmin (alpha$_2$-AP), and thrombin-activatable fibrinolysis inhibitor (TAFI). All such molecules bind among themselves (enzymes and inhibitors) and to the fibrin clot: the final result is the balance of the prevailing activities. tPA and plasminogen both bind to the fibrin surface where tPA is an effective catalyst of plasminogen activation. Initially, plasmin proteolysis of fibrin generates new, higher affinity binding sites for plasminogen, providing an amplifying loop of plasminogen activation. To reinforce this process, generated plasmin converts scuPA, an ineffective catalyst, to the efficient two-chain uPA, thereby increasing the local concentration of efficient plasminogen activators. Anti-fibrinolytic molecules oppose these events: alpha$_2$-AP, both soluble and cross-linked to fibrin, forms complexes with plasmin, thus rendering it inactive. PAI-1 rapidly reacts with both tPA and uPA, reducing the concentration of plasminogen activators. Formation of activated TAFI (TAFIa) results in removal of plasmin-generated COOH-terminal lysine residues, thus suppressing the rate of fibrin lysis (for details, see section B of this Figure). Fibrin degradation occurs by cleavage at the D-E-D domains (shown in section B) of fibrin polymers by plasmin to yield the variety of polymers illustrated in the Figure . B: mechanisms of thrombin activatable fibrinolysis inhibitor (TAFI) effects toward fibrinolysis. Thrombin-thrombomodulin (IIa-TM) on the surface of endothelial cells cleaves TAFI to its active carboxypeptidase form (TAFIa). TAFIa interferes with fibrinolysis by cleaving COOH-terminal arg (R) or lysine (K) residues made available as a result of partial plasmin digestion of the fibrin clot. Removal of these residues attenuates the self-amplifying mechanism of fibrin-dependent plasmin formation, where partial plasmin proteolysis of fibrin increases the number of binding sites (COOH-terminal lysines) available for efficient plasminogen activation.

Another important disease where inflammation-dependent fibrinogen accumulation has been shown to play a pathogenic role is multiple sclerosis (MS). In MS, early signs of inflammatory demyelination include entry of fibrin(ogen) into the central nervous system (CNS), which is normally excluded by the blood-brain barrier, and up-regulation of the PA system. Up-regulation of plasminogen activators and of metallo-proteases in MS is considered to play a major role in the disturbance of the blood-brain barrier and subsequent leukocyte entry leading to inflammation, as well as causing myelin breakdown (140, 141). Significant up-regulation of uPAR and PAI-1 are among the earliest detectable signs of inflammatory demyelination (142). Fibrin(ogen) enters the CNS before clinical disease and demyelination (143, 144). tPA, which is constitutively expressed in CNS (145), is co-localized with fibrin and damaged axons in MS lesions, suggesting an attempt of the fibrinolytic system to dissolve fibrin deposits (146). However, excess PAI-1 prevents tPA activity in MS, thereby contributing to fibrin deposition and axonal injury (147). Fibrin deposition has been shown to hinder axonal regeneration in a model of peripheral nerve

damage (148), and removal of fibrin in experimental allergic encephalomyelitis (EAE) suppressed disease development and reduced neurological deficit (149, 150). The frequency of fibrin deposits has suggested the possibility of a "fibrin perspective" in CNS pathologies (151). Fibrin(ogen) has the ability to exacerbate inflammation acting through signalling molecules to modulate cell adhesion and migration: leukocyte engagement of fibrin(ogen) via the integrin receptor alphaM-beta2/Mac-1 is critical for host inflammatory response (152); fibrin(ogen) recognition also regulates leukocyte transendothelial migration (153). Additionally, fibrin(ogen) engagement of leukocyte Mac-1 activates transcriptional regulation of the IL-1 beta promoter (154) and macrophage chemokine secretion through toll-like receptor 4 (155). On these basis, inhibition of PAI-1 in order to promote fibrinolysis has been proposed as a potential target for therapeutic intervention in MS (156).

8. CONCLUSIONS

In this review we have discussed the main results indicating a pathogenic role of the plasminogen activation system in inflammation, according to its effects in each one of the historical cardinal signs of inflammation (*rubor, calor, tumor, dolor*) as a sort of tribute to the continuity of scientific knowledge. Nevertheless, there are cutting edge observations indicating new and unexpected roles of the plasminogen activation system in specific inflammatory pathologies, which put the basis for further development and therapeutic interventions. The uPA/uPAR interaction is involved in triggering of immune complex (IC)-dependent diseases. The formation of IC and their tissue deposition induces an acute inflammatory response termed the Arthus reaction. Allergic broncho-pulmonary aspergillosis, chronic obstructive lung, and farmer's lung are pulmonary conditions that feature the Arthus reaction. The initial events after a challenge by IC include activation of two effector pathways responsible for the development of inflammation: the complement system and IgG Fc-gamma-Rs on resident effector cells, such as macrophages. IC induce the formation of the bioactive C5a anaphylotoxin that, in turn, interacts with the C5a receptor (C5aR) which induces up-regulation of IgG Fc-gamma-Rs on resident effector cells. Such cells, upon interaction with IC, initiate synthesis and release of pro-inflammatory cytokines (IL-1, TNFalpha), which initiate and perpetuate the inflammatory response. The uPA/uPAR system is activated in the lung and particularly in resident alveolar macrophages (AM) early in IC-triggered alveolitis. The presence of uPAR is necessary for an adequate C5a/C5aR signalling in AM, and uPA occupancy of uPAR synergistically increases C5a-induced effects in these cells, mainly for up-regulation of IgG Fc-gamma-Rs and subsequent synthesis of inflammatory cytokines (157). Similarly, uPA/uPAR interaction mediates a pro-inflammatory effect in human glomerular mesangial cells (MC) via expression of the anaphylatoxin C5a receptor (158). MC are central to the pathogenesis of progressive glomeruli-associated renal diseases. Upon up-regulation of C5aR, MC proliferate, secrete cytokines and growth factors and activate transcription factors and early response genes (159). All these parameters determine the degree of mesangial injury, which in turn determines the final outcome of the kidney inflammatory process.

The PA system continues to surprise the scientific community with plenty of unexpected activities in every field of human pathology. Evolution of medicine has clearly shown that inflammation is a useful reaction aimed to control tissues injury, but also that it may be potentially dangerous to the function of the inflamed organ or tissue. We believe that times are becoming mature to try application of enhancers or of inhibitors of plasminogen activation activity and of receptor expression as new therapeutic tools to promote the inflammatory process as well as to control inflammation-related injury.

9. ACKNOWLEDGEMENTS

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