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Invited Review

Vessel wall-dependent metabolic pathways of the adhesive proteins, von-Willebrand-factor and vitronectin

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Summary. The integrity of the vessel wall under quiescent conditions as well as its appropriate responsiveness under conditions of stimulation, inflammation or vascular injury is controlled by a number of adhesive interactions involving distinct cellular receptor systems and various multifunctional adhesive ligands. While a number of these extracellular matrix components of the vessel wall are endogenously produced, secreted and deposited, exogenous adhesion proteins may become translocated from the intra- to the extravascular space by virtue of endothelial cell-specific transport systems. Two prominent examples for each metabolic pathway are discussed. Endothelial cell-specific biosynthesis and secretion as well as deposition of multimeric von-Willebrand-factor within intracellular granules (Weibel-Palade bodies) relates to the first possibility of processing, whereas binding of reactive forms of circulating vitronectin to diverse cellular receptors with subsequent extravasation and deposition into the extracellular matrix appears to be characteristic for the second case. In this review the known features of the metabolic routes of both adhesion proteins are discussed and set in perspective to their functional properties. Their localized mode of action in the vessel wall appears to be crucial for balanced haemostasis and immune systems, two major defence mechanisms of the organism.

Key words: von-Willebrand-factor, Vitronectin, Vessel wall, Metabolic pathway, Multimerization

1. Introduction

The maintenance of tissue integrity of the vessel wall under quiescent conditions relies on intact adhesive interactions between cells and between cells and the extracellular matrix (ECM)-network involving cellular receptors and counter receptors as well as tight anchorage to extracellular adhesive components. Spatial and temporal expression and rearrangement of adhesion receptors and the provision of cell-derived ECM, enables the vessel wall to fulfill its various functions as active and dynamic filtration barrier and to maintain the vascular tone and its non-thrombogenic properties under undisturbed conditions. Vessel wall injury with the exposure of wound areas results in a fast, selective and localized recruitment of blood cells to the inflamed site and requires new coordination of adhesive interactions. Tight adhesion and transmigration of neutrophils into inflammatory extravascular sites, selective phagocytosis of cell remnants by macrophages or granulocytes, or the initial platelet adhesion and aggregation to the exposed subendothelium are prominent examples in this respect. A number of adhesive glycoproteins which are present in the subendothelial ECM, released from platelet α-granules upon aggregation or circulating as plasma proteins, are involved in these interactions: fibronectin serves a major adhesion function in the vessel wall and fibrinogen constitutes the major polymerizing substance of a wound-sealing thrombus. Von-Willebrand-factor (vWF) is essential for the initial platelet adhesion to the exposed vessel wall ECM and protects coagulation factor VIII during transport in the blood stream. Vitronectin stabilizes cell-ECM interactions and mediates fast clearance and uptake of end-products of the coagulation and complement cascade, thereby acting as multifunctional host protection factor. Thus, besides their prominent adhesive functions, the metabolic pathways particularly of the latter two proteins relate to their functional role and are crucial for the homeostasis of the vessel wall. Detailed descriptions of structure-function relationships of vWF and vitronectin are found in recently published reviews (Tomasini and Mosher, 1990; Wagner, 1990; Preissner, 1991). Before discussing and distinguishing the metabolic routes for vWF and vitronectin, some general aspects of adhesive interactions related to the vascular system will be

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summarized.

2. Vessel wall-related adhesive mechanisms

During the last decade, the discovery of several distinct super gene families of adhesion molecules (e.g. integrins, selectins, immunoglobulins, cadherins and respective ligands) has led to new insights into mechanisms of cell-cell and cell-ECM interactions which are important not only for the functional integrity of the vessel wall but also for its responsiveness in defence processes. In order to resist mechanical forces and shear stress, monolayer cell-cell contacts (such as for endothelial cells) have to be maintained for a considerably long period of time in a quasi-static fashion involving homophilic or heterophilic binding between receptors and counter-receptors, respectively, on adjacent cells. Furthermore, integrins, proteoglycans, and other cell surface adhesion receptors have been implied to establish the firm contacts between the basolateral face of endothelial cells and the underlying basal lamina as well as to warrant tight anchorage of smooth muscle cells and fibroblasts with their pericellular ECM. Modulations of cell-cell and cell-ECM contacts by change in expression of adhesion molecules, by pericellular proteolysis, or by simply not allowing firm interactions to occur (such as selectin-mediated neutrophil rolling on intact endothelium) result in variable and dynamic short-term adhesion or detachment events which control cell migration and invasion (Humphries, 1990; Yamada, 1991).

Prominent examples for the versatility of a single class of adhesion receptors are the transmembraneous heterodimeric «integrins», which serve to form integral cell contacts in focal adhesions by linking cytoskeleton-associated cytoplasmic components on one side and ECM-proteins on the other side (Hynes, 1987; Ruoslahti, 1991). Moreover, integrins have been implied to mediate cell migration on various substrata, relevant for the formation of new blood vessels during angiogenesis or necessary for movement of smooth muscle cells from the medial to the intimal layer of the vessel wall (Hynes, 1992). Extracellular ligands are initially recognized through their Arg-Gly-Asp (RGD) sequence or related motifs by integrins in a divalent cation-dependent manner (Humphries, 1990; Yamada, 1991). In addition, cell-bound complement C3bi and several transmembrane proteins such as Ig-like cell adhesion molecules (ICAMs) and vascular cell adhesion molecule (VCAM) serve as cell-surface ligands for some integrins (Hemler, 1990). The low affinity of a single receptor-ligand interaction is overcome by clustering of integrins on an immobilized, multivalent substratum of ECM proteins such as collagen, fibronectin, vitronectin, laminin or others. Cell adhesion is strengthened by the ability of individual integrins to display overlapping ligand recognition or to be promiscuous in binding several adhesive proteins. Except for mature erythrocytes, all cell types in the body express these receptors, and the high compositional diversity within the gene family is reflected by the variable α/ß subunit composition of each integrin. Dependent on the kind and extent of contact with a given ECM, cells may not only respond by altering their morphological appearance, but can also undergo differentiation (Lin and Bissell, 1993). Conversely, differentiated (endothelial) cells which have lost their survival-dependent contacts to the ECM may ultimately enter into apoptosis (Meredith et al., 1993). Thus, the integrin-dependent change in the phenotype of a cell is intimately linked to its metabolic machinery, and the involvement of integrins in signal transduction pathways has been proposed (Kornberg et al., 1991; Hynes, 1992). Among others, a central cytoplasmic factor in these events appears to be «focal adhesion kinase» (pp125FAK), which becomes phosphorylated at tyrosine upon cell spreading (Schaller et al., 1992) and which is found together with cytoskeleton-associated components such as vinculin, talin, paxillin or tensin on the intracellular fase of focal adhesions (Burridge and Bockholt, 1993). This «outside-in» signalling is differentiated from «inside-out» signalling in platelets, where a non-integrin agonist (such as thrombin) mediates cytoplasmic rearrangement and subsequent «activation» or affinity modulation of αIIbß3-integrin, resulting in fibrinogen-dependent platelet aggregation (Juliano and Haskill, 1993; Williams et al., 1994).

Vessel wall-derived proteoglycans are a heterogeneous class of complex glycosaminoglycan-decorated core proteins which function as structural components within the ECM, and heparansulphate proteoglycans are able to locally concentrate growth factors and proteases (Brunner and Preissner, 1994). Cell membrane-associated proteoglycans (either transmembraneous or glycolipid-anchored) provide part of the pericellular glycocalix, contribute to basolateral anchorage of endothelial cells, or mediate recognition and uptake of macromolecules from the luminal side, respectively. In addition, the endothelium is equipped with a number of «scavenger» or «clearance» receptors that facilitate removal of altered, aged or oxidized protein (complexes) (Schnitzer, 1993).

3. Multifunctional adhesive glycoproteins: vWF and Vitronectin

Except for platelets which circulate in mammalian organisms as non-nucleated cells and thereby rely on their given repertoire of adhesion glycoproteins and receptors, adhesive cells of the vasculature respond to extracellular stimuli by induction or repression of adhesion receptor gene expression. Respective adhesion receptor ligands in the vessel wall, (1) may be produced and secreted locally or (2) become accumulated through translocation processes and thereby contribute to integrin regulation by providing a specific pericellular environment. Endothelial cell- and megakaryocyte-specific biosynthesis and processing of vWF is an example for the first possibility, whereas extravasation

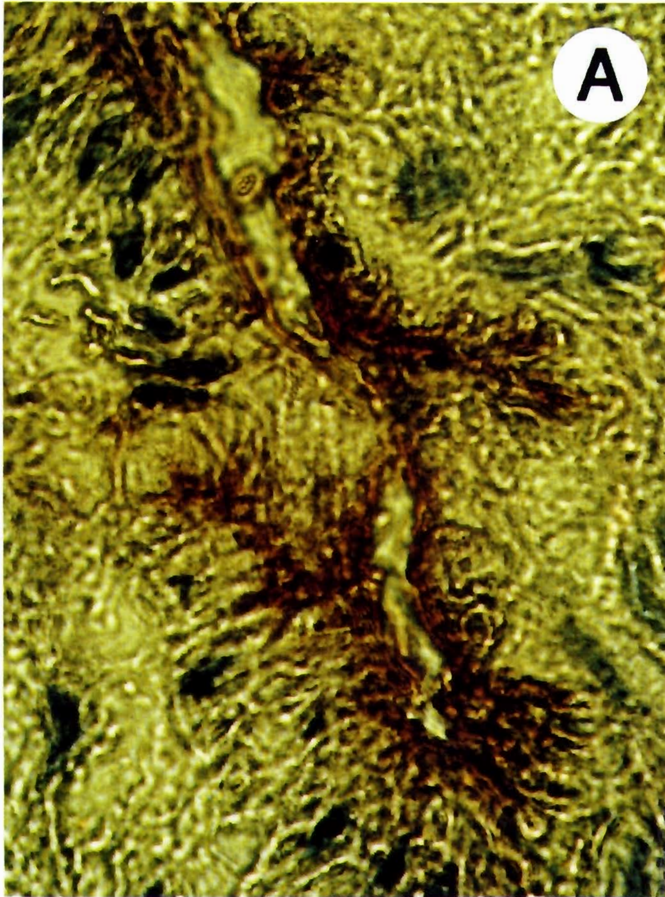
and subsequent deposition of circulating vitronectin appears to be characteristic for the second case.

3.1 Biosynthesis, molecular structure and function of vWF

vWF is an exception in the family of adhesive proteins, because of its multimeric structure which relates to vWF's highly specialized function in haemostasis as well as to its intracellular processing and storage within endothelial cell-specific granules, designated Weibel-Palade bodies (Fig. 1). vWF circulates in normal human plasma as a series of disulphide bonded multimers ranging in size from 0.5-20 x 10^3 kDa and is found together with other adhesive proteins in platelet α-granules (Ruggeri and Zimmerman, 1981). The human vWF gene has been localized to chromosome 12 (Verweij et al., 1986), and the complete exon/intron structure of the vWF gene which spans about 0.1% of this chromosome has been established (Sadler et al., 1991). The 52 exons are arranged in four different homologous domains termed (from the amino- to the carboxy-terminus) D1, D2, D3, D', D3, A1, A2, A3, D4, B1, B2, B3, C1, C2 and become translated only in endothelial cells (Jaffe et al., 1974) and megakaryocytes (Nachman et al., 1977). At present, very little information is available about this remarkable tissue specificity and the high levels of expression of vWF, although 2.2 kb of the upstream untranslated region have been analyzed, where a typical TATA box around position -30 has been identified (Sadler et al., 1991). The vWF subunit is synthesized as a 2,813 amino acid precursor polypeptide with a short signal peptide followed by an unusual long 741 amino acid propeptide, encompassing about 25% of the total protein. The primary translation product undergoes several posttranslational modifications (including dimerization, N- and O-glycosylation, sulphation, multimerization, propeptide cleavage and subsequent storage or secretion) in the endoplasmic reticulum and Golgi apparatus. During this processing more than 50 intra- and interchain disulphide bridges are formed which are particularly important for (a) dimerization between carboxy-termini of peptides and for (b) multimerization of mature vWF dimers between amino-termini (Lyons and Ginsburg, 1994). Moreover, regulatory proteins retain vWF-subunits until dimerization via disulphide bond formation has occurred (Bonthron et al., 1986), and an acidic environment within the post-Golgi compartment as well as the concentration of calcium ions are essential for correct multimerization (Mayadas and Wagner, 1991) indicating an active catalytic process in oligomerization of dimers.

The pro-vWF-polypeptide appears to be essential for multimer assembly, since COS cells transfected with vWF cDNA lacking the propiece only produced dimers (Verweij et al., 1987). Furthermore, processing of uncleavable pro-vWF as well as transfection of COS cells with separate vectors containing the cDNAs for pro-vWF-polypeptide and the mature subunit, respectively, resulted in secretion of vWF with normal multimer pattern (Wise et al., 1988). Due to sequence homology of both D-domains in the pro-vWF-polypeptide with catalytic regions in protein disulphide isomerases (which normally contribute to protein folding and oligomerization in the endoplasmic reticulum), a putative disulphide isomerase activity within vWF would provide an independent mechanism for vWF multimerization in post-endoplasmic reticulum compartments (Mayadas and Wagner, 1992). During multimerization a subtilisin-type protease removes the propeptide at the correct cleavage site. Although this pro-piece contains an RGD-site, an active collagen-binding domain and exhibits sequence homology to chemotactic polypeptides (Kao et al., 1993), its possible functions remain obscure.

vWF is essential for platelet adhesion and aggregation at sites of vascular injury, and plasma, endothelial cell, and platelet vWF contribute to the formation of a platelet plug (Tschopp et al., 1974). vWF multimers offer the possibility for multivalent interactions between a thrombogenic surface and platelet receptors, and particularly under high shear stress of bood flow no other adhesive protein can substitute for vWF in this respect. The large vWF multimers exhibit high affinity binding to subendothelial matrix structures and fibrillar collagens type I, III or collagens type IV and VI may be involved. Two types of receptors, the platelet surface glycoprotein complex GPIb/IX and the αIIbß3 platelet integrin establish the initial contact between platelets and the injured vessel wall or mediate vWF-binding to activated platelets leading to spreading and aggregation, respectively (Savage et al., 1992). Although constitutively exposed on non-activated platelets, GPIb does not recognize circulating vWF, unless the adhesive ligand has undergone conformational transition(s) due to ECM binding or its high local concentration at vessel wall matrix sites. In vitro, addition of the antibiotic ristocetin or the snake venom protein botrocetin as well as neuraminidase-treatment of vWF renders the ligand susceptible for binding to platelet GPIb. These alterations apparently mimic the conformational transition of vWF at ECM structures necessary for platelet adhesion in vivo. The binding site for GPIb has been localized within the structure of the A1-domain loop where other functional regions, including binding sites for collagen, heparin, sulphatides and botrocetin, are present (Mohri et al., 1988). Fragments of this portion have been successfully used as antithrombotic agents thereby preventing initial vWF-platelet interaction (Gralnick et al., 1992). The other adhesive domain containing the RGD recognition site is located towards the carboxy-terminus of vWF within the C1 domain and mediates binding to activated platelets via integrin αIIbß3 or promotes endothelial cell attachment via integrin αVß3 (Dejana et al., 1989). Like fibrinogen, multimeric vWF serves a similar bridging function by interacting with activated platelets during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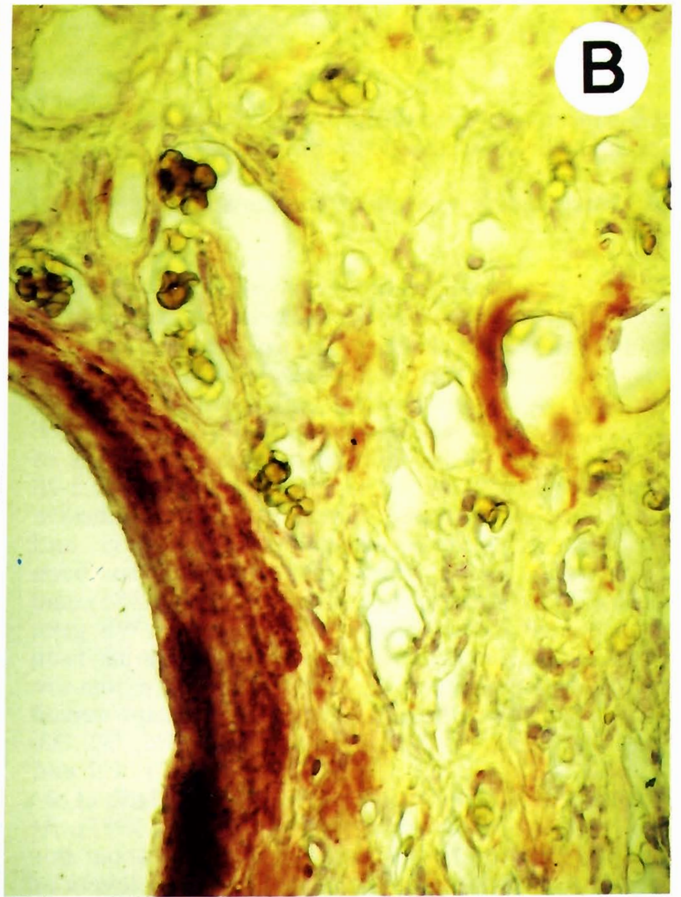
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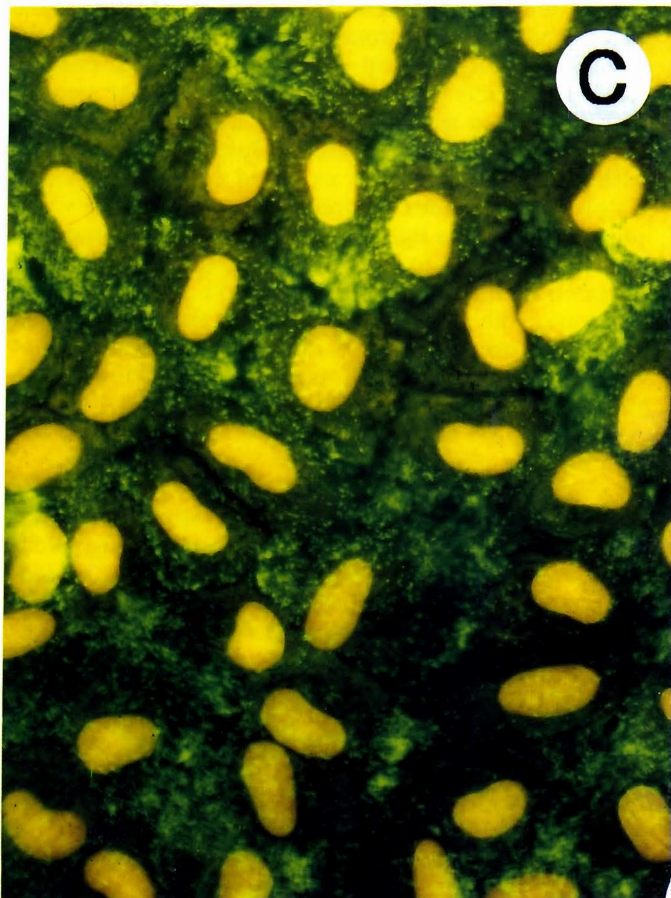
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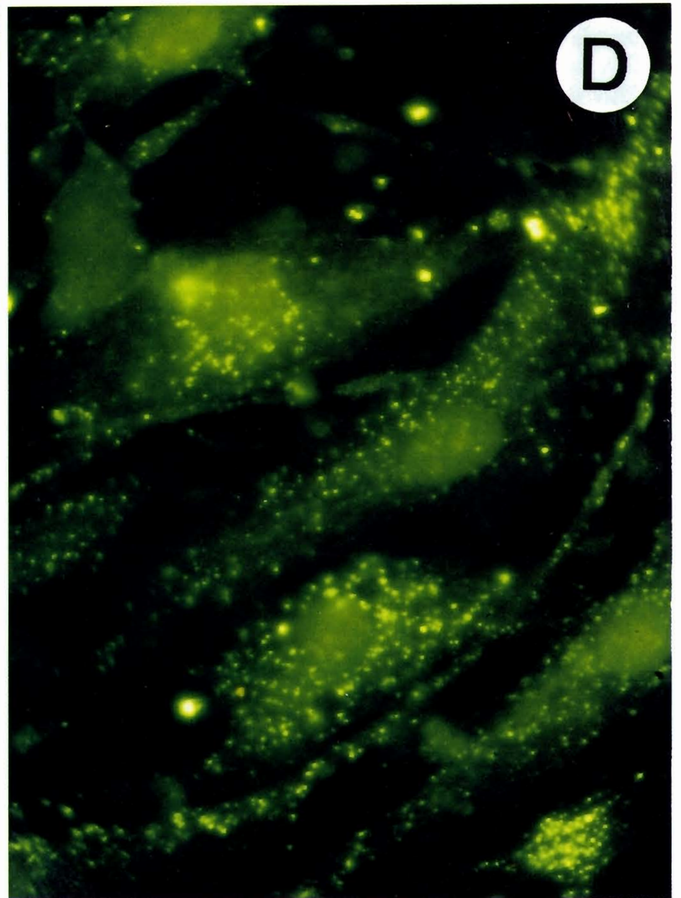
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their aggregation process. Subsequent to thrombin activation of platelets, GPIb/IX bound vWF may become internalized (Hourdillé et al., 1992), while αIIbß3 integrin is shuttled from α-granules to the cell surface where these receptors become activated. This bi-directional translocation of vWF-receptors may strengthen the transition from initial platelet adhesion towards platelet aggregation.

The physiological importance of these structural features stems from diagnosis of patients with inherited abnormalities of vWF which are found with a frequency of 1-3% in the population. Although von-Willebrand's-disease (vWD) is an extremely heterogeneous disorder with more than 20 distinct clinical subtypes described, two major classes can be distinguished: the most common, type I vWD, is characterized by a quantitative disorder with vWF levels between 20 and 50% of normal, whereas in type II patients the synthesis of defective vWF protein is observed which often relates to the absence of high and intermediate size vWF multimers (Fig. 2). Typical clinical symptoms of the autosomal dominant type I vWD are a prolonged bleeding time with skin or gastrointestinal bleeding and menorrhagia. Although one could assume that mutations affecting regulatory elements in the vWF-gene or the biosynthetic processing and intracellular degradation of defective vWF molecules may lead to reduced secretion, the underlying molecular mechanisms of type I vWD have not yet been clarified. The treatment of choice in these cases is intranasal administration of the vasopressin analogue D-amino-8-D-arginino vasopressin (DDAVP) which generally results in marked elevation of vWF released from its storage pools in endothelial cells, the Weibel-Palade-bodies, and thereby serves to prevent fatal bleeding complications. In type II vWD, several missense mutations have been identified (Ginsburg and Bowie, 1992) characterized e.g. by the loss of multimer formation, higher susceptibility of multimers to degradation, defective intracellular transport, or defects in the binding site for GPIb on platelets.

In addition to its adhesive functions, endothelial cell-derived plasma vWF serves to transport coagulation factor VIII in a non-covalent complex and thereby protects this important cofactor against early proteolytic activation and inactivation. Through binding to the amino-terminal region of mature vWF, factor VIII becomes localized to sites of platelet plug and fibrin clot formation, underlining the critical role of vWF in blood clotting. Patients with type I vWD and those with mutations in the factor VIII binding domain of vWF are typically presented with decreased levels of factor VIII activity, exhibiting characteristics of haemophilia A when factor VIII activity is found below 5% of normal (Ginsburg and Bowie, 1992).

3.2 Metabolic pathways of vWF

vWF is secreted from endothelial cells by two different and independent routes: a constitutive and a regulated pathway (Fig. 3A). In an experimental system, about 90% of vWF takes the first route and becomes secreted, while about 10% remains packaged as high molecular weight multimers in specialized organelles. The existence of a regulated pathway was deduced from the in vivo observations that exercise or injection of epinephrine or vasopressin caused a rapid rise in plasma levels of vWF compatible with the release of vWF from intracellular storage compartments. Histocytochemical analysis of cultured endothelial cells with antibodies specific to vWF show a strong granular staining pattern with intracellular rod-shaped structures with dimensions of 0.1 by 4 µm (Weibel and Palade, 1964; Wagner et al., 1982) (Fig. 1). These Weibel-Palade bodies originate from the trans-Golgi apparatus (Sengel and Stoebner, 1970), and experiments using different vWF-mutants demonstrate that the pro-vWF-polypeptide contains the signal sequence which induces formation of these organelles and packaging of vWF therein (Wagner et al., 1991). Interestingly, other secretory cells transfected with the pro-vWF-polypeptide start to produce Weibel-Palade body-like structures indicating that the signal is universally recognized by those cells which contain a regulated pathway of secretion (Voorberg et al., 1993). Although the stoichiometry of the free pro-polypeptide and the mature subunits in Weibel-Palade bodies is 1:1, proteolytic processing of vWF and multimerization preceeds the formation of these organelles (Vischer and Wagner, 1994). Besides vWF only small amounts of the lysosomal protein CD63 as well as P-selectin were found in isolated Weibel-Palade bodies (Bonfati et al., 1989), and the latter one follows the regulated secretory pathway in an experimental system (Koedam et al., 1992). P-selectin is a transmembrane protein of the selectin family and mediates adhesion of neutrophils and monocytes to activated endothelial cells (Johnston et al., 1989). Due to rapid translocation from Weibel-Palade

Fig. 1. Immunohistochemical analysis of the distribution of vWF and vitronectin. **A.** The staining of vWF in endothelial cells of the intimal layer of an artery is shown using polyclonal antibodies against vWF followed by alkaline phosphate-anti-phosphatase enhancement. **B.** Staining of medium calibre brain artery by monoclonal antibodies against vitronectin followed by alkaline phosphatase-anti-phosphatase enhancement is shown (experiment carried out by Dr. H. Opitz, University of Tübingen, Germany). Note the strong association of vitronectin with the medial layer and the subendothelial basement membrane, whereas other extravascular tissue regions remain negative. **C.** Indirect immunofluorescent staining of a confluent monolayer culture of human umbilical vein endothelial cells using antibodies against vWF. Note the intense staining of Weibel-Palade bodies, the unique vWF storage granule; the counter staining was performed with ethidium bromide. **D.** While cultured arterial smooth muscle cells appear to be negative for the expression of vitronectin, prominent granular staining (using fluorescent-labelled antibodies against vitronectin) is found following specific binding of exogenous multimeric vitronectin to cellular receptors (experiment carried out by Dr. S. Hess, MPI, Bad Nauheim, Germany). Similarly, endothelial cells bind and endocytose reactive multimeric forms of the adhesive protein. A, B: x 400; C, D: x 1,000

bodies in endothelial cells or α-granules in platelets and subsequent integration into the cell surface membrane, P-selectin serves as a sensitive marker for early cellular activation.

vWF is released from Weibel-Palade bodies by exocytosis, and under in vitro conditions secretion of vWF is induced by thrombin, fibrin, histamine, the complement components C5b-9, oxygen radicals, and calcium ionophore or phorbol esters (Wagner, 1990). Although it is known that Weibel-Palade body-associated secretion coincides with calcium influx and requires microtubule-contraction, the signal cascade leading to regulated vWF release remains obscure. In particular, it is unclear whether receptors such as the thrombin receptor or integrins are involved in this process. Studies on endothelial cells cultured on filters showed that stimulation with physiological secretagogue induced vWF release in both basolateral as well as luminal direction, and it is very likely that under in vivo conditions a similar bidirectional vWF release does occur. In contrast, the calcium ionophore A23187 induces vWF release exclusively to the basolateral side of endothelial cells. The existence of an intracellular positive feed-back mechanism can be ruled out, since release of vWF from Weibel-Palade bodies is not associated with increased rates of vWF-de-novo synthesis.

The constitutive pathway differs in two respects from the regulated one: firstly only dimers and smaller vWF-multimers are secreted constitutively and secondly, this pathway shows a strong polarity. Endothelial cells cultured on filter membranes under quiescent conditions

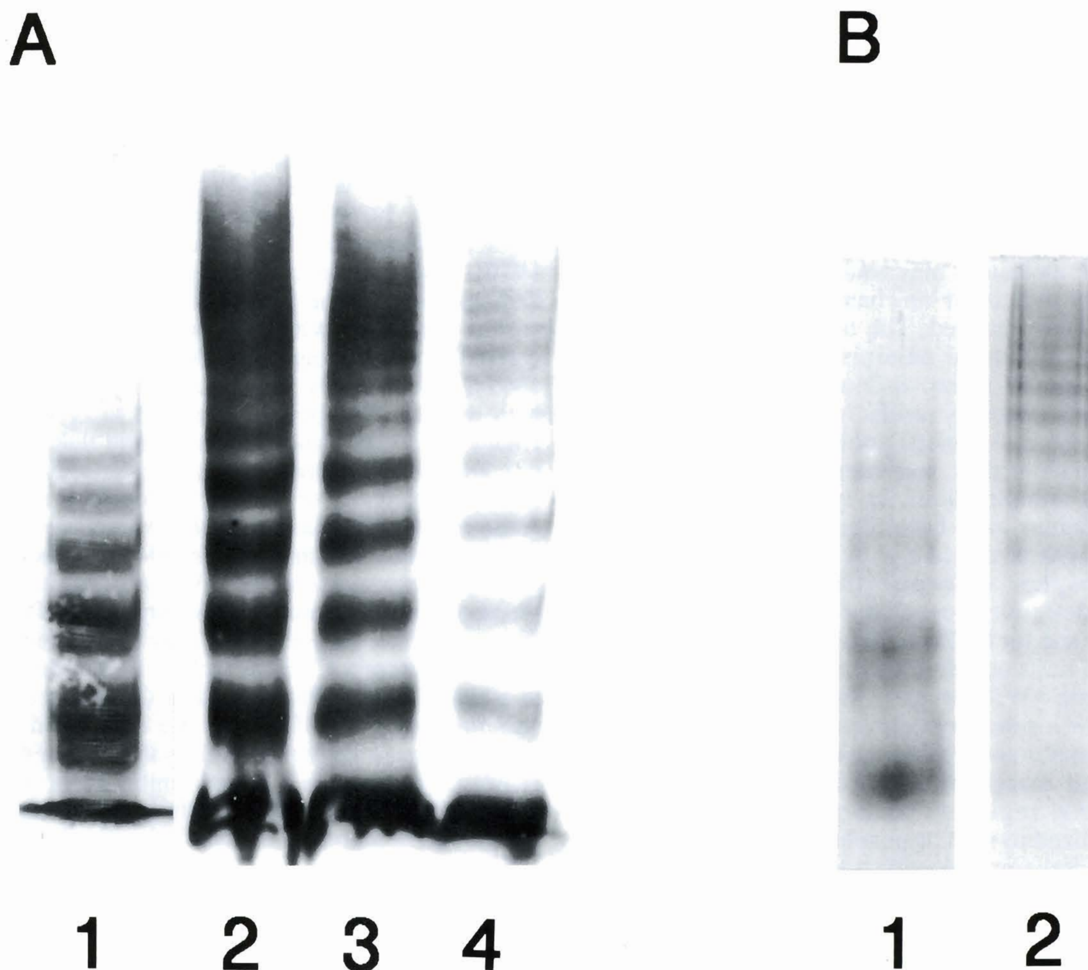

Fig. 2. Analysis of multimeric forms of vWF and vitronectin by gel electrophoresis. **A.** Human plasma samples from two healthy individuals (lanes 2, 3) or plasma from patients with type I vWD (lane 4) and typ II vWD (lane 1) were subjected to agarose gel electrophoresis in the presence of 0.1% sodium dodecyl-sulphate, followed by transfer of proteins onto nitrocellulose membrane. The vWF-specific band pattern (from dimers at the bottom/anode to high molecular weight multimers at the top/cathode) was visualized by reaction with peroxidase-labelled antibodies against vWF followed by chemiluminescence development. Note the decreased level of vWF protein in lane 4 and the missing multimers in lane 1. **B.** Analysis of purified human plasma vitronectin on polyacrylamide gel-electrophoresis in the absence of sodium dodecyl-sulphate before (lane 1) and after multimerization (lane 2) induced by 6M urea. Note that monomeric and (disulphide-bonded) dimeric plasma vitronectin tend to self-associate into multimers once the conformation of the protein becomes disturbed by e.g. chaotropes (Stockmann et al., 1993).

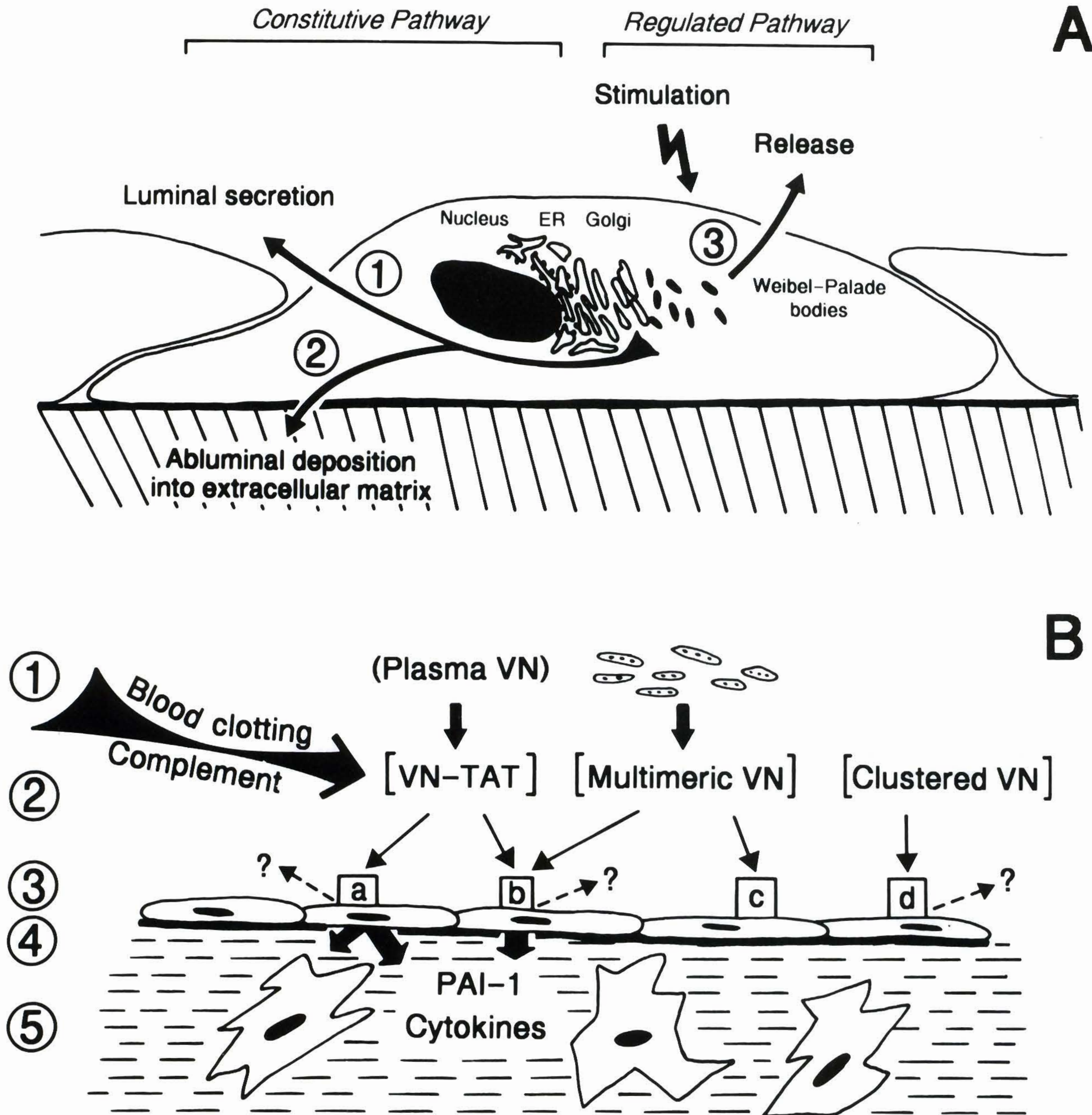

Fig. 3. Schematic representation of metabolic pathways for vWF and vitronectin. **A.** Following expression of vWF in endothelial cells with subsequent post-translational modifications (see text) in the endoplasmic reticulum (ER) and the trans-Golgi apparatus, two metabolic routes are taken; the constitutive and the regulated pathway. In the first pathway, the major portion of mature vWF subunits dimerizes between carboxy-termini and eventually forms oligomers between amino-termini of dimers (both reactions involve a catalytic process leading to formation of disulphide bridges) before they become secreted towards the luminal face of the monolayer (1) or are deposited towards the basolateral side in the basement membrane/ECM (2). In the second pathway the minority of vWF dimers are forced to covalently self-associate into high molecular weight multimers in a pro-peptide-dependent manner followed by cleavage of the pro-sequence. vWF multimers become stored in post-golgi compartments, designated Weibel-Palade bodies; their processing mechanism is largely unknown. Dependent on the nature of a stimulus, vWF-multimers are released in a polarized fashion (3) and thereby contribute to the initiation of early platelet plug formation. **B.** Upon activation of the blood coagulation and complement systems as well as during vascular injury reactive multivalent forms of vitronectin are generated from the latent plasma form or are released e.g. by platelets (1). Reactive species include ternary complexes such as vitronectin-thrombin-antithrombin III (VN-TAT) or multimeric and clustered vitronectin (2). While plasma vitronectin binds poorly to endothelial cell monolayers, these products are recognized by a variety of yet unidentified receptors including possibly proteoglycans (3a), scavenger-type receptors (3b) or others (3c) as well as integrins (3d). Following binding, translocation of VN-TAT and multimeric VN onto the basolateral side of the cells and subsequent deposition into the ECM is observed (4). In this microenvironment a number of localized functional activities are mediated by vitronectin including stabilisation of plasminogen activator inhibitor-1 (PAI-1), binding of cytokines, induction of cell migration, complement inhibitory function and possibly others. Vessel wall located vitronectin thereby constitutes a multifunctional host protection factor in humoral defence mechanisms.

secrete vWF exclusively to the luminal aspects but not to the basolateral side. The intracellular selection mechanisms discriminating between vWF release or storage have not yet been identified. As a major component of the α-granules in megakaryocytes and platelets vWF is released via the regulated pathway (Cramer et al., 1985) and participates in platelet aggregation.

3.3. Biosynthesis, molecular structure and function of vitronectin

Human vitronectin is found as a single chain polypeptide with M_r=78,000 in the circulation and is associated with different ECM sites. Liver cells predominantly synthesize vitronectin (Barnes and Reing, 1985), and there is no indication for alternative splicing mechanisms during processing (Jenne and Stanley, 1987). Only a limited number of cell types such as mesothelial cells, monocyte/macrophages, megakaryocytes, and some transformed cell lines express this adhesive protein, whereas fibroblastic cells as well as vascular endothelial cells, smooth muscle cells or other non-hepatic cells do not produce vitronectin.

Several immunofluorescent and histochemical studies suggest the deposition of vitronectin in a fibrillar pattern in some connective tissues (Hayman et al., 1983), in association with dermal elastic fibres in skin (Dahlbäck et al., 1986) as well as with renal tissue (Falk et al., 1987) and the media of the vascular wall (Niculescu et al., 1987; Reilly and Nash, 1988; Guettier et al., 1989) (Fig. 1). Moreover, the accumulation of terminal complement complexes (C5b-9 and/or SC5b-9) along elastic fibres later in life and the association of vitronectin with keratin bodies during keratinocyte programmed cell death (apoptosis) (Dahlbäck et al., 1989) as well as colocalization of vitronectin with deposits of the terminal complement complex in kidney tissue from patients with glomerulonephritis (Falk et al., 1987; Bariety et al., 1989) suggest a role for vitronectin in preventing tissue damage in proximity to local complement activation. The deposits of vitronectin in fibrotic and necrotic regions of arteriosclerotic blood vessels (Niculescu et al., 1987; Guettier et al., 1989; Sato et al., 1990) point to a possible but yet uncharacterized role of vitronectin in the pathogenesis of arteriosclerotic lesions. These data demonstrate that appreciable amounts of vitronectin may become deposited at sites distant from actual biosynthesis of the protein. It remains to be established whether these pathological sites throughout the organism are the cause or the consequence for subsequent vitronectin accumulation. The interaction of vitronectin with glycosaminoglycans (Lane et al., 1987) or different types of native collagens (Gebb et al., 1986) as well as crosslinking of vitronectin by transglutaminase/factor XIIIa (Sane et al., 1990) are reactions that are likely to occur at ECM sites in vivo as well.

The molecular structures of human, rabbit and mouse vitronectins have been reported (Jenne and Stanley, 1985; Suzuki et al., 1985; Sato et al., 1990) indicating more than 80% sequence homology. Other vitronectin homologues or vitronectin-like factors have been recently described in a number of invertebrate and plant organisms, but until additional sequence information is available, their identity remains unclear (Kitagaki-Ogawa et al., 1990). The exon/intron organization of the human vitronectin gene (on chromosome 17) (Jenne and Stanley, 1987) and the mouse counterpart (Seiffert et al., 1993) are identical. Using promotor constructs with various sites of the 5' untranslated region fused to a reporter gene, interleukin 6-responsive elements were proposed in the mouse gene, supported by about 3-fold induction of vitronectin biosynthesis in the liver upon acute-phase response in vivo (Podor et al., 1993). In addition, transforming growth factor ß leads to a similar increase in vitronectin production in hepatoma cells (Koli et al., 1991). Besides its prominent adhesive properties, vitronectin forms stable ternary complexes with thrombin-serine protease inhibitor complexes and binary complexes with plasminogen activator inhibitors and may thereby control the distribution of anti-proteolytic activity in the pericellular microenvironment (Preissner and Jenne, 1991). Vitronectin also functions as inhibitor of cytolytic reactions of the terminal complement pathway as well as of perforin in cytolytic T-cells and thereby prevents autologous cell lysis. Localization of binding sites, which include (from the amino- to the carboxy-terminus) those for plasminogen activator inhibitor 1 (PAI-1), integrins, collagens, heparin, complement components, and perforin, plasminogen and PAI-1, was mostly established by in vitro binding experiments and points to the multifunctional role of this adhesive protein in defence mechanisms. A complete genetic deficiency of vitronectin has not been described; acquired deficiencies with up to 50% reduction of vitronectin plasma levels have been diagnosed in several patients suffering from disseminated intravascular coagulation and degenerated liver diseases (Kemkes-Matthes et al., 1987; Conlan et al., 1988).

Stabilized by heteropolar interaction, the predominant form of vitronectin in plasma appears to exist as an internally folded molecule, also supported by electron-microscopical analysis (Stockmann et al., 1993). Hydrodynamic data have suggested a conformational lability/flexibility of vitronectin (Preissner and Müller-Berghaus, 1987; Tomasini and Mosher, 1988; Jenne et al., 1989) reflected by a transition of vitronectin from this «closed» into an «extended» form. This process may be provoked by physiological ligands such as the thrombin-antithrombin III or the complement C5b-7 complex or by surface coating and denaturation, and concomitant to unfolding, additional cryptic binding sites such as for heparin or the initial inducers become exposed. The RGD-dependent attachment site of vitronectin appears to be unaffected by these transitions. The distant sequence homology between vitronectin and

the heme-binding plasma protein hemopexin (Jenne and Stanley, 1987) whose ligand-binding properties strongly depend on conformational flexibility as well (Smith et al., 1988), extends to other members of this «hemopexin-type» family such as matrix-metalloproteinases.

3.4 Metabolic pathways of vitronectin

The conformational transition from the latent plasma form into heparin-binding isoform(s) of vitronectin which tend to self-associate into multimers, serves as the key event in the generation of reactive species of the adhesive protein. The ternary vitronectin-thrombin-antithrombin III and the vitronectin-C5b-9 complex show markedly increased levels in patients with septicemia or thrombotic complications, respectively (de Boer et al., 1993), and their short half-lives in the circulation are very likely due to tight complexation with the multimeric form of the adhesive protein. These high molecular weight complexes acquire integrin-dependent cell attachment promoting activity when used as immobilized substrates (Preissner et al., 1988) and vitronectin endows them with additional recognition sites for alternative cellular receptors. In a heparin-dependent manner, soluble multimeric vitronectin or vitronectin-containing complexes were recognized by saturable binding sites on endothelial cell monolayers (de Boer et al., 1992; Hess et al., 1993), and heparan sulphate proteoglycans have been implied, at least in part, to be responsible for this interaction. In addition to heparin, other polyanionic compounds such as fucoidan or poly-inosinic acid, which are effective competitors for scavenger type receptors, were able to inhibit binding of multimeric vitronectin to endothelial cells, indicating the involvement of these clearance receptors in recognition of altered forms of the adhesive protein as well. Whether the scavenger receptor on macrophages, which may function as adhesion receptor in a divalent cation-independent manner for serum components (Fraser et al., 1993), mediates adhesion to vitronectin remains to be established.

In vitro experiments have established the putative mechanism(s) by which reactive forms of circulating vitronectin reach extravascular sites: in an energy-dependent process the multivalent ligand is translocated either into lysosomal compartments of endothelial cells or becomes transcytosed and appears at the basolateral aspect in association with the basement membrane (de Boer et al., 1992; Hess et al., 1993). Based on ultrastructural data, a coated-pit initiated pathway which may diverge intracellulary is involved in uptake and processing of vitronectin (complexes). The constant supply of the vessel wall-associated ECM with reactive vitronectin may thereby explain the (age-related) accumulation of the protein along large vessels or elastic fibres in skin (Dahlbäck et al., 1989).

Besides multimeric vitronectin, high molecular weight aggregates have been generated in vitro by heat denaturation and served as a model for a high-density, reactive form of the adhesive protein released from platelets or associated with (tumor) cell surfaces. These aggregates resembled vitronectin, covalently immobilized to microspheres, and both modified forms were recognized by luminally expressed integrins on endothelial cell monolayers (Conforti et al., 1992), since RGD-peptides as well as antibodies against αVß3-integrin blocked this association (Zanetti et al., 1994). This surprising result is indicative for a switch in receptor recognition dependent on the «density» of the adhesive ligand and appears to be similar to integrin recognition of clustered, immobilized ligands. Furthermore, discrimination between integrin - reactive and - nonreactive forms of adhesion factors allows the prevention of unwanted spontaneous adherence of blood cells to the vessel wall and thereby warrants in part the non-thrombogenic properties of the undisturbed vascular endothelium. High density forms of vitronectin may be able to cluster integrins on the luminal phase of the endothelium and thereby transmit cellular signals. Further studies have to define the mechanisms and consequences of these events.

In the ECM environment vitronectin is able to mediate cell adhesion both in an integrin- and a proteoglycan-dependent mechanism, since both ligand-binding domains are apparently exposed (de Boer et al., unpublished observation). Furthermore, ECM-anchorage of vitronectin is mediated by binding to native collagens via at least two binding site), and direct interactions with morpho-regulatory proteins such as osteonectin or tenascin (Rosenblatt et al., 1993) may contribute to rearrangement of cellular interactions. Whether the anti-adhesive properties of these factors or their exposure towards cellular receptors are directly counteracted by vitronectin remains to be determined. Moreover, direct in vitro binding experiments with cultured vascular smooth muscle cells revealed contribution of both integrins and proteoglycans in recognizing multimeric forms of vitronectin (Hess et al., unpublished observation). Although the adhesive protein is able to directly bind to growth factors such as transforming growth factor ß or platelet derived growth factor BB (Schoppet et al., unpublished results) and mediates chemotactic as well as haptotactic activities (Naito et al., 1991), it is unclear whether these properties are expressed in the vessel wall environment.

During early haemostatic plug formation effective control of endothelial cell-derived plasminogen activators is obtained through endothelial cell-derived PAI-1 (Loskutoff et al., 1989) in order to warrant initial thrombus formation and stabilization and to prevent bleeding. PAI-1 is a major synthesis and release product of vascular cells, but requires vitronectin as ECM-associated binding and stabilizing protein in the intact vessel wall (Preissner et al., 1990), and both components are also co-localized in areas of arteriosclerotic lesions (Lupu et al., 1993). Polarized secretion and the quantities of deposited PAI-1 strongly correlate with

available ECM-associated vitronectin (Grulich-Henn et al., 1992). Due to its broad distribution, the vitronectin-PAI-1 complex appears to be of general importance for the control of pericellular proteolysis and to stabilize cell-ECM contacts (Ciambrone and McKeown-Longo, 1990). Fixation of PAI-1 by vitronectin in the ECM not only results in a drastic prolongation of the half-life of the inhibitor (Mimuro and Loskutoff, 1989) but also changes the specificity of PAI-1 such that (ECM-associated) thrombin becomes a target enzyme as well exhibiting, however, a 100-fold slower inhibition kinetic compared to t-PA (Ehrlich et al., 1990). This relationship appears to be beneficial for the dynamic switch from blood clotting to fibrinolysis during haemostasis, since mutual neutralization of the procoagulant key enzyme thrombin and the fibrinolytic key inhibitor PAI-1 occurs. Conversely, high PAI-1 deposits in the vessel wall are likely to postpone the necessary switch such that restenosis may affect the vessel patency, whereas administration of antibodies to PAI-1 reduce thrombus extension and promote low-dose t-PA-mediated thrombolysis (Levi et al., 1992). It can further be hypothesized that vitronectin-bound PAI-1 in the medial smooth muscle ECM may serve to limit the mitogenic properties of thrombin which are related to the proteolytic activation of the functional thrombin receptor on these cells (Bar-Shavit et al., 1990).

Binding of PAI-1 to vitronectin is mediated by a two-domain interaction involving both the amino- and the carboxy-terminus of the adhesive protein (Preissner et al., 1990; Seiffert and Loskutoff, 1991) and a region in PAI-1 also responsible for fibrin and heparin binding (Lawrence et al., 1994). Modulation of these interactions can occur by: (a) partial proteolysis of vitronectin (Chain et al., 1991; Sane et al., 1991; Kost et al., 1992); (b) competitive binding to other ECM-associated proteins such as osteonectin; (c) non-enzymatic modification of critical residues in vitronectin by glucose metabolites (resulting in advanced glycosylation end products) (Preissner et al., 1993). Initial plasmin-catalyzed limited proteolysis produces a vitronectin fragment which lacks both heparin and major PAI-1 binding sites leading to liberation/mobilization of the inhibitor within the vessel wall (Kost et al., 1992; Gechtman et al., 1993). Moreover, this intermediate fragment exhibits sequence homology/identity in its carboxy-terminus to respective sites in α_2-plasmin inhibitor or α-enolase critical for plasminogen binding. In accordance with these in vitro data, in areas of skin inflammation associated with bullous pemphigoid the majority of blister-fluid derived vitronectin was present as the plasminogen-binding fragment and co-localized with plasmin(ogen) at sites of the dermal-epidermal junction (Kramer et al., 1993). Taken together, these findings indicate that intact ECM-associated vitronectin acts as anti-fibrinolytic adhesive cofactor, whereas limited proteolysis by plasmin converts vitronectin into a pro-fibrinolytic, plasminogen-binding cofactor. This process not only limits the thrombus-stabilizing activity of the vitronectin-PAI-1 complex but also allows the destabilization of cellular adhesive interactions necessary for cell migration or invasion to occur (Kost et al., 1992).

4. Concluding remarks

Two major adhesive proteins relevant for haemostatic mechanisms are co-localized in the ECM of the vessel wall, but quite different metabolic routes guide vWF and vitronectin to their destinations. Although a number of details regarding biosynthesis, multimerization and deposition of vWF in endothelial cell-specific Weibel-Palade bodies have been characterized, the molecular mechanisms which lead to partitioning of this regulated pathway and the constitutive pathway of vWF-processing remain largely unknown. In addition, putative functional properties of the pro-vWF-polypeptide, which is generated in stoichiometric amounts when compared to mature vWF, need further investigation. Elucidation of these topics will certainly lead to a general insight into the processing of regulated pathways of secretion in other cells as well. The contrasting features of vitronectin deposition in the vessel wall certainly need additional effort in order to characterize respective receptor systems for recognition and uptake as well as their comparison with established scavenger receptor pathways which are known to remove various reaction end products and altered macromolecules from the blood circulation. It is still obscure whether multivalent forms of vitronectin are involved in mediating cell-cell contacts and in which way vitronectin's unique binding properties for the chemokine ß-endorphin (Hildebrand et al., 1989) are involved in this process. Identification of vitronectin-receptor interactions at various sites in the body may thereby facilitate the analysis of vitronectin function in normal and diseased tissues or during development.

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