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Invited Review

Constitutive and regulated expression of vitronectin

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Summary. Tissue homeostasis depends on spatially and temporally controlled expression of multifunctional adhesive glycoproteins and their cellular counter receptors, and on a tight regulation of proteolytic enzyme systems. The adhesive glycoprotein vitronectin (Vn) not only regulates adhesive events, but also controls a number of these proteolytic enzyme cascades, including the complement, coagulation, and fibrinolytic systems. However, understanding of the biological functions of this molecule is complicated due to it's conformationally lability and its tendency to self-associate. While plasma Vn is monomeric and lacks exposure of conformationally sensitive epitopes, platelet and tissue-associated Vn are believed to be conformationally altered and multimeric. The latter forms express a functional repertoire distinct from plasma Vn. While little Vn immunoreactivity is detectable in most normal tissues, increased depositions of Vn have been observed in areas of tissue injury and necrosis. Tissue Vn was believed to be plasma-derived, but recent studies indicate that extrahepatic cells have the biosynthetic potential to produce Vn and that its synthesis can be regulated under inflammatory conditions. Here, the constitutive and regulated expression of Vn, its locations in tissues and interaction with other matrix molecules are reviewed and their implications for the functions of this molecule are discussed.

Key words: Vitronectin, Extracellular matrix, Cell adhesion, Proteolysis

Introduction

Vitronectin (Vn) belongs to the group of adhesive glycoproteins that play key roles in the attachment of cells to their surrounding matrix and may participate in the regulation of cell differentiation, proliferation, and morphogenesis (for reviews, Tomasini and Mosher, 1990; Preissner, 1991). Vn is identical to the S-protein of the complement system (Jenne and Stanley, 1985; Suzuki et al., 1985; Tomasini and Mosher, 1986). In this system, it functions as inhibitor of cytolytic reactions of the terminal complement complex as well as perforin in cytolytic T-cells (Tomasini and Mosher, 1990; Preissner, 1991), by that preventing cell lysis. In addition, Vn also appears to serve several regulatory functions in the coagulation and fibrinolytic systems. For example, Vn binds thrombin/serine protease inhibitor complexes, and thus may be involved in the clearance of "spent" molecules either by the liver or through transcytosis into extracellular matrices (Ill and Ruoslathi, 1985; Jenne et al., 1985; Podack et al., 1986; Preissner et al., 1987; Tomasini and Mosher, 1988; De Boer et al., 1992). Vn functions as an inhibitor of heparin-stimulated reactions of antithrombin III with blood coagulation factors II and X (Akama et al., 1986; Lane et al., 1987; Preissner and Mueller-Berghaus, 1987). In addition, it binds to and stabilize the biological activity of type 1 plasminogen activator inhibitor (Mimuro et al., 1987; Declerk et al., 1988; Mimuro and Loskutoff, 1989; Seiffert and Loskutoff, 1991a,b), the physiological inhibitor of both tissue-type and urinary-type plasminogen activators (Loskutoff et al., 1989; Loskutoff, 1991; Van Meijer and Pannekoek, 1995). Moreover, Vn modulates the substrate specificity of type 1 plasminogen activator inhibitor, by that endowing this molecule with thrombin inhibitory functions (Ehrlich et al., 1990, 1991). Taken together, these observations suggest that Vn provide a unique regulatory link between cell adhesion and proteolytic enzyme cascades. Although initially described as a plasma protein, recent studies provide evidence that Vn is also present in various tissues, and that its biosynthesis may be regulated under pathophysiological conditions. These studies are reviewed and their implications for the biological function(s) of Vn are discussed.

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Regulation of Vn functions by its conformational state

During the last few years, it has been increasingly appreciated that Vn is a conformationally labile molecule. This conclusion is based on the observation

that only 2% of the Vn in plasma is in a conformation capable of binding to heparin-Sepharose, whereas the relative amount increases to 7% by the generation of serum (Izumi et al., 1989). In addition, complex formation of Vn with type 1 plasminogen activator inhibitor (Seiffert and Loskutoff, 1996a,b), thrombin-antithrombin III (Tomasini and Mosher, 1988; Tomasini et al., 1989), and complement C5b-C9 (Hogasen et al., 1992), induces conformational changes in the Vn molecule, leading to the exposure of the cryptic C-terminal heparin binding domain (located between amino acids 341 and 379). Moreover, conformational changes in the Vn molecule can also be induced by denaturation with chaotropic agents, heat-treatment and acidification, and these changes are accompanied by the spontaneous formation of disulfide-linked Vn multimers (reviewed in Tomasini and Mosher, 1990; Preissner, 1991). Using a panel of conformationally sensitive antibodies, we provided evidence that conformational changes in the Vn molecule are not limited to the C-terminal heparin binding domain, but also occur in the N-terminal half of the molecule (amino acids 1 to 239), including the N-terminal somatomedin B (amino acids 1 to 51) domain (Seiffert, 1995).

Plasma Vn and Vn purified under non-denaturing conditions (i.e., native Vn) are monomeric and lack exposure of conformationally sensitive epitopes (Tomasini and Mosher, 1988; Seiffert, 1995; Seiffert and Schleef, 1996). In contrast, Vn purified from serum by heparin affinity chromatography in the presence of 8M urea (i.e., denatured Vn) is highly multimerized and conformationally altered (Yatohgo et al., 1988; Hogasen et al., 1992; Bittorf et al., 1993; Stockmann et al., 1993). A second circulatory pool of Vn is contained in platelet α-granules (Parker et al., 1989; Preissner et al., 1989; Asch and Podack, 1990; Stockmann et al., 1993). Platelet Vn is present in both monomeric and multimeric forms and the latter forms express epitopes for conformationally sensitive antibodies (Stockmann et al., 1993; Seiffert and Schleef, 1996).

Defining the biological functions of Vn has been complicated because a number of its ligand-binding domains are differentially expressed according to the conformation of this molecule. A number of ligands were identified that interact preferentially with the conformationally altered, denatured form of Vn. These ligands include collagen (Gebb et al., 1986), glycosaminoglycans (Barnes et al., 1985; Hayashi et al., 1985; Akama et al., 1986; Izumi et al., 1989), ß-endorphin (Hildebrand et al., 1988, 1989), type 1 plasminogen activator inhibitor (Naski et al., 1993), and urokinase/urokinase receptor complex (Wei et al., 1994; Waltz and Chaphan, 1994). In contrast, thrombin-antithrombin III complexes appear to preferentially interact with native Vn (de Boer et al., 1992). The situation is even more complex in the case of cell adhesion. Radiolabeled native Vn was reported to bind specifically and saturable to stimulated platelets in a glycoprotein IIb/IIIa-dependent manner (Thiagarajan and Kelly, 1988a,b; Asch and Podack, 1990), whereas reports on the binding of denatured Vn are conflicting (DiFaxio et al., 1994). In addition, Vn has been reported to both block (Rogers et al., 1995) and stimulate (Asch and Podack, 1990) platelet aggregation. The reasons for this apparent discrepancy remain presently unclear.

Vitronectin binds to specific cell surface receptors, collectively termed integrins (for review, Ruoslahti and Pierschbacher, 1987; Hynes, 1992). Each integrin is a non-covalently associated α,ß heterodimer that spans the plasma membrane. To date, nine integrin α-subunits and twelve ß-subunits have been identified. Since the ligand binding specificity of each receptor is conferred by its subunit composition, functional diversity within the family of integrins is achieved by heterologous pairing between subunits. In this respect, the platelet integrin glycoprotein IIb/IIIa (Thiagarajan and Kelly, 1988a,b), αvß3, expressed by endothelial cells and implicated in angiogenesis (Brooks et al., 1994; Drake et al., 1995), and αvß5 (Smith et al., 1990), and α8ß1 (Schnapp et al., 1995) have been identified as Vn-binding integrins. Vitronectin contains a single RGD-sequence (amino acids 45 to 47) in close proximity to the type 1 plasminogen activator inhibitor and urokinase/urokinase receptor complex binding sites (Jene and Stanley, 1985; Suzuki et al., 1985; Seiffert and Loskutoff, 1991a,b; Seiffert et al., 1994a,b). Site-directed mutagenesis studies reveal that this sequence is required for cell adhesion to Vn and can not be compensated for by other parts of the molecule (Cherny et al., 1993; Zhao and Sane, 1993).

The cell adhesion domain is believed to be exposed on both native and denatured Vn and not to be effected by the conformational changes of Vn (Preissner, 1991; Zanetti et al., 1994). However, this concept is challenged by three observations. Firstly, only denatured Vn induces tyrosine phosphorylation in endothelial cells in an αvß3-dependent manner (Bhattacharya et al., 1995). Secondly, αvß5-dependent endocytosis of Vn by skin fibroblasts is only observed using denatured Vn (Panetti and McKeown-Longo, 1993a,b; Panetti et al., 1995). Thirdly, denatured Vn binds to and compete with the binding of labeled denatured Vn to glycoprotein IIb/IIIa and αvß3 in a dose-dependent manner, whereas little binding/competition of native Vn is detectable (unpublished observation). Moreover, type 1 plasminogen activator inhibitor induced Vn multimers interact with purified integrins (unpublished observation), suggesting that Vn ligands elaborated locally under pathophysiological conditions may convert Vn in an adhesive glycoprotein. It should be noted that conformational changes and multimerization are closely linked processes (Hogasen et al., 1992; Bittorf et al., 1993; Stockmann et al., 1993). It thus remains unclear whether the observed functional differences with respect to binding to integrins are mediated by conformational changes, by multimerization events, or a combination of both. Independent of the elucidation of the exact mechanism, these results strongly suggest that native Vn is not an adhesive

glycoprotein.

Vitronectin in body fluids

Vitronectin is present in normal plasma at concentrations of 200 to 400μg/ml (Barnes et al., 1983; Shafer et al., 1984; Preissner et al., 1985; Conlan et al., 1988), and thus constitutes 0.2 to 0.5% of total plasma proteins. The concentration of plasma Vn does not significantly differ from that in serum prepared either by recalcification of plasma or whole blood (Tomasini and Mosher, 1990; Preissner, 1991; unpublished observation). Thus, although purified Vn is a substrate for factor XIIIa and tissue transglutaminase (Sane et al., 1990, 1991; Skorstengaard et al., 1990), little if any Vn becomes covalently incorporated into ex vivo prepared blood clots. Reduced plasma levels of Vn have been reported in patients with severe liver failure (Kemkes-Matthes et al., 1987; Conlan et al., 1988). In these patients, changes in plasma Vn levels closely parallel change in biosynthetic markers of the liver parenchyma (Conlan et al., 1988; Inuzuka et al., 1992; Hogasen et al., 1996). These observations suggest that the liver is the major source of plasma Vn. Increased plasma levels of Vn have been detected in patients undergoing elective orthopedic surgery (Seiffert et al., 1995a,b) and in rodents undergoing acute phase reactions (Seiffert et al., 1994a,b, 1995a,b). Taken together, these latter results suggest that Vn is an acute phase protein (see below).

A second circulatory pool of Vn is contained within platelets in a rapid releasable form, which accounts for approximately 0.8% of the circulating pool of Vn (Parker et al., 1989; Preissner et al., 1989; Asch and Podack, 1990; Stockmann et al., 1993). It is unknown whether Vn in platelets is synthesized by megakaryocytes or endocytosed from plasma and incorporated into α-granules. Whereas plasma Vn is monomeric and lacks significant exposure of epitopes for conformationally sensitive monoclonal antibodies (see above), platelet Vn has been observed to be partially present in a high molecular weight, conformationally altered form (Stockmann et al., 1993; Seiffert and Schleef, 1996). Platelet Vn is also functionally distinct from plasma Vn with respect to glycosaminoglycan binding (Seiffert and Schleef, 1996). More specifically, while little binding of plasma Vn to heparin Sepharose is detectable, significant amount of the platelet form of Vn bind to glycosaminoglycans. Platelet activation is apparently not required for the generation of multimeric Vn in platelets (Seiffert and Schleef, 1996). Immunohistochemical studies reveal that Vn is present in platelet α-granules (Roger et al., 1992). Similar conclusions are derived from functional studies (parallel release with α-granule marker proteins upon platelet stimulation [Preissner et al., 1989]) and from biochemical subcellular fractionation experiments (Seiffert and Schleef, 1996). The ability of platelets to adhere during thrombosis and increase their local concentration up to 200-fold as compared to the circulating level (Leung et al., 1983) suggest that this multimeric form of Vn will be released at the platelet-platelet, platelet-cell, or platelet-matrix interface, thereby expressing a functional repertoire distinct from the form in plasma.

Vn also has been found in urine, amniotic fluid, cerebrospinal fluid, and bronchoalveolar lavage fluid (Shaffer et al., 1984; Pohl et al., 1991; Eklund et al., 1992; Teschler et al., 1993; and unpublished observations). Although the concentration of Vn in amniotic fluid is relatively low, its specific activity (defined as μg Vn/mg total protein) is identical to plasma (Shaffer et al., 1984). The specific activity of Vn in urine is twice that of the plasma (Shaffer et al., 1984). Although Vn is present in the lavage fluid from healthy volunteers, the amount is approximately 10-fold higher in patients with interstitial lung disease, including pulmonary fibrosis, sarcoidosis, and idiopathic pulmonary fibrosis (Pohl et al., 1991; Eklund et al., 1992; Teschler et al., 1993). These results raise the possibility that Vn may serve to regulate inflammatory reactions in the alveoli associated with lung disease. Interestingly, Vn enhances internalization of asbestos fibers by pleural mesothelial cells via the integrin $\alpha v\beta 5$, raising the possibility that Vn may be involved in asbestos-induced mesothelioma formation (Boylan et al., 1995). Cultured alveolar macrophages secrete a protein immunological identical to Vn (Petterson et al., 1990), suggesting that the Vn in the lavage fluid may be synthesized locally by alveolar macrophages. This conclusion is consistent with in situ hybridization studies of normal murine lung tissue (see below). In general, there is no age-dependent difference in the Vn concentration in any of these body fluids. However, fetal cord blood contained only 60 to 70 percent of the Vn present in adult blood (Shaffer et al., 1984).

Constitutive expression of vitronectin

Only a limited number of cultured cells have been shown to synthesize and secrete Vn. It is important to evaluate published reports for the possibility that the Vn has been endocytosed from the medium and released during subsequent incubation steps. Indeed, the later has been recently demonstrated for human umbilical endothelial cells (Voelker et al., 1993; De Boer et al. 1995). Definitive proof of Vn biosynthesis, based for example on metabolic labeling followed by immunoprecipitation with Vn-specific antibodies, has only been provided for two human hepatoma cell lines (Hep G2 and Hep 3B; [Barnes and Reing, 1985; Suzuki et al., 1985; Seiffert et al., 1990]). However, data is available to suggest that other cells also produce Vn, including blood-derived cultured monocytes and macrophages (Hetland et al., 1989), and a teratoma cell line resembling parietal endoderm (Cooper and Pera, 1988, 1993).

During the last few years, our knowledge about cell types that produce Vn in vivo has drastically increased. Vn cDNAs have been isolated from two independent

human liver libraries, indicating that Vn mRNA is present in the liver (Jenne and Stanley, 1985; Suzuki et al., 1985). The same holds true for the rodent liver (Sato et al., 1990; Seiffert et al., 1991). In situ hybridization reveals that the hepatocyte is the major Vn producing cell type in the murine liver (Seiffert et al., 1991), a finding that has been confirmed in rats by liver cell fractionation studies (Seiffert et al., 1995a,b). Detection of Vn mRNA in extrahepatic organs depends highly on the detection limit of the assay method employed. Northern blotting studies reveal that Vn mRNA is undetectable in mouse heart, lung, kidney, spleen, muscle, brain, thymus, uterus, testes, skin, adipose tissue, and aorta (Seiffert et al., 1991). It should be noted that Solem et al reported in addition low levels of Vn-specific mRNA in mouse brain using the Northern blotting approach (Solem et al., 1991).

With the development of more sensitive RNA detection methods, it became obvious that Vn mRNA is present in most murine organs. Using quantitative competitive polymerase chain reaction, we reported the presence of Vn mRNA in practically every major murine organ, the only possible exemption appears to be the blood (Seiffert et al., 1994a,b). Although the liver contains the highest concentration of Vn mRNA, significant levels are also detected in the brain (25-fold less than liver) and in adipose tissue, heart, and skeletal muscle (100-fold less than liver) (Seiffert et al., 1994a,b). Lower concentrations also are detected in the lung, uterus, testis, and thymus. Little Vn mRNA can be detected in kidney and spleen (Seiffert et al., 1994a,b). These results indicate that significant amounts of Vn mRNA are produced in extrahepatic murine organs. In all cases, the levels are clearly higher than the levels that might be expected from the leakiness (i.e., ectopic expression) of eukaryotic promoters (Chelly et al., 1989; Sarkar and Sommer, 1989).

The widespread expression of Vn mRNA in murine tissues raises the possibility that a common cell type in all these organs (for example vascular cells) synthesizes this molecule. Recent studies using in situ hybridization reveal that the extrahepatic expression of Vn mRNA in some murine tissues is localized to subsets of specific cell types, and that these cell types frequently produce Vn mRNA at levels approaching that of the hepatocytes (Seiffert et al., 1996a,b). For example, in the central nervous system, high levels of Vn mRNA are prominent in arachnoid cells and in cells frequently associated with brain capillaries, whereas neuronal cell populations are in general negative (Seiffert et al., 1996a,b). In the myocardium, the signal is localized to cells in the endomyocardium and subepicardial fat. Additionally, the pulmonary alveolar walls contain Vn-positive cells. The parenchyma of the kidney and spleen are negative within the detection limit of our in situ hybridization procedure (Seiffert et al., 1996a,b). We attempted to quantify the in situ hybridzation results based on the exposure time required to first detect the positive signal. In the central nervous system and liver, a positive signal is detectable within 3 days of exposure. Although only a small subset of cells is positive in the brain, the signal intensity of these cells is similar to that of the liver. In the heart, positive cells are detected within 1 week of exposure, while approximately 8 weeks of exposure are required to detect a signal in the lung (Seiffert et al., 1996a,b). These results indicate that a small subset of cells present in extrahepatic tissues can express Vn at levels approaching those of the hepatocytes.

The expression of Vn mRNA in extrahepatic organs is not limited to the adult organism. In situ hybridization analysis of murine embryonic tissues reveal Vn mRNA primarily in the liver and the central nervous system (Seiffert et al., 1995a,b). In the liver, Vn mRNA is detected as early as day 10, the levels increasing at later developmental stages. In the central nervous system, Vn mRNA is also detected as early as day 10, and is confined to the floor plate. However, as development proceeds, high levels of Vn transcripts become prominent in the meninges of the cortex and the spinal cord, and in close proximity to brain capillaries. In contrast, Vn mRNA is not associated with blood vessels in peripheral organs. These results indicate that Vn is expressed in a spatially and temporally distinct pattern during murine embryogenesis. It should be noted that gene targeting experiments reveal that Vn is not required for normal murine development (Zheng et al., 1995). However, the animals have not yet been characterized under pathophysiological conditions.

Our knowledge regarding the expression of Vn in human tissues is rather limited. For example, Gladson et al. reported the expression of Vn in advanced human astrocytoma by in situ hybridization and immunohistochemistry, whereas Vn mRNA was undetectable by in situ hybridization in low grade tumors or normal adult brain (Gladson and Cheresh, 1991; Gladson et al., 1995). It remains unclear whether the absence of Vn mRNA in human brain is due to species differences between humans and rodents, or whether the in situ hybridization procedure employed in our studies using murine tissue was more sensitive in comparison to the human study. Vn mRNA was also detected in the male genital tract (Nuovo et al., 1995; Sawada et al., 1996). Here, Vn was transcribed exclusively in the germ cells at the spermatocyte and round spermatid stages. Vn mRNA was also detected in stromal cell cultures derived from human colorectal adenocarcinomas (Tomasini-Johansson et al., 1994), raising the possibility that Vn may be produced by these tumors in situ. Similar findings have been reported for ductal adenocarcinomas of the pancreas (Loehr et al., 1994). Thus, while little is known about the expression of Vn in normal human tissues, a number of tumor cells have the biosynthetic potential to synthesize Vn and may be causally related to the disease process.

Regulated expression of vitronectin

Vitronectin biosynthesis is not only regulated in a

spatially and temporally distinct pattern during murine development, but also in adult rodents. We studied the regulation in a murine model system in which acute systemic inflammation was induced by intraperitoneal endotoxin administration (Seiffert et al., 1994a,b). Plasma Vn levels increase 2 to 3-fold within 16 hours after endotoxin administration and remain elevated up to 72 hours. This increase appears to result from increased synthesis in the liver, since the steady-state level of hepatic Vn mRNA increases 4-fold after endotoxin injection in a time- and dose-dependent manner. Moreover, Vn mRNA levels in heart, lung, and brain are not significantly changed by endotoxin, suggesting that endotoxin regulate Vn gene expression in a tissue-specific fashion (Seiffert et al., 1994a,b). Similar results are obtained in rats livers in which the acute phase reaction is induced by endotoxin, turpentine, or Freund's adjuvant (Seiffert et al., 1995a,b). To analyze the individual effects of raised interleukin 6, a major acute phase cytokine, and glucocorticoids on Vn gene expression in vivo, rats were also injected with either dexamethasone or purified recombinant rat interleukin 6. Vn mRNA increases in the liver within 1 hour after interleukin 6 injection, raising the possibility that the rat Vn gene is directly responsive to interleukin 6. Also chronic injection of low levels of interleukin 6 resulted in increase hepatic Vn mRNA levels (Seiffert et al., 1995a,b). In contrast, rats injected with dexamethasone show unchanged Vn expression. Taken together, these results indicate that the Vn gene is up-regulated in acute and chronic inflammation, and this induction is primarily mediated by interleukin 6. The cis-acting elements involved in the acute phase regulation of the murine Vn gene have been identified (Seiffert et al., 1996a,b). The acute phase response is well preserved throughout phylogeny and is believed to play an important protective role in host defense against tissue damage and infection. Many of the acute phase proteins act as antiproteinases, opsonins, or blood-clotting and wound-healing factors, which may protect against generalized tissue destruction associated with inflammation. Vn shares several functional similarities with classical acute phase proteins, including regulatory functions in the complement, blood coagulation, and fibrinolysis. The physiological role of the upregulation of Vn should probably be viewed in the context of these functions.

Regulation of Vn gene expression is not limited to rodent species. For example, plasma concentrations of Vn increase approximately twofold following elective orthopedic surgery and remain elevated up to 5 days (Seiffert et al., 1995a,b). This observation suggests that the human Vn gene may also be acute phase responsive. It should be noted that cultured human hepatoma cells are unresponsive to interleukin 6 (Seiffert et al., 1996a,b), whereas transforming growth factor-α results in an up-regulation of the Vn transcript and secreted protein (Koli et al., 1991; Seiffert et al., 1996a,b). These observations are confirmed in experiments in which the 5'-flanking region of the human Vn promoter is fused to the luciferase reporter gene and transiently transfected into Hep-3B human hepatoma cells (Seiffert et al., 1996a,b). While transforming growth factor-α stimulates the human reporter gene construct, interleukin-6 is ineffective. Control experiments using a murine Vn reporter gene construct reveal that the cell line is responsive to interleukin-6 (Seiffert et al., 1996a,b). It remains unclear at present whether the human Vn gene is sensitive to interleukin-6 in vivo or has developed transforming growth factor-α responsiveness in order to acquire the possibility of being regulated under pathophysiological conditions.

Deposition of vitronectin into tissues

Immunolocalization studies of Vn have been performed using a variety of polyclonal and monoclonal antibodies. This raises the possibility that the antibodies recognize uncharacterized cross-reacting molecules. For example, it has been reported that Vn antigen is present in embryonic tissue, fetal membranes, smooth and skeletal muscle, kidney, supporting stroma of portal triads, and the capsular surface of visera (Hayman et al., 1983). However, the monoclonal antibody employed in this study has been later found to cross-react with a 30kD extracellular matrix protein distinct from Vn (Tomasini-Johansson et al., 1993), thus questioning the significance of these findings. A second problem with immunolocalization studies for Vn is related to the abundance of this molecule in the vascular system. Specifically at lower magnifications, it may be hard if not impossible to localize the Vn specific signal to vascular structures or the extravascular tissue itself. Ideally, adjacent tissues should be stained with antibodies that recognize plasma proteins not to be expected to be present at extravascular sites. These stringent staining controls have only been employed in a limited number of studies.

The skin is maybe the most studied tissue, and Vn is found using both monoclonal and polyclonal antibodies associated with dermal fibrillar structures (Hintner et al., 1991; Dahlbaeck et al., 1993). Interestingly, Vn immunoreactivity is invariably detected in conjunction with elastic fibers in specimens from individuals over 13 years of age, whereas Vn staining is faint or absent in patients between the age of 6 and 13 years (Dahlbaeck et al., 1989). Vn is also associated with apoptotic keratin bodies in the skin (Hintner et al., 1989). Membrane-bound aggregates of keratin intermediate filaments, if not rapidly phagocytosed, loose their enclosing membrane and are found in the dermis. Increased formation of keratin bodies occurs in response to UV-light (Hintner et al., 1989), and consistently, increased Vn staining can be detected in sun-exposed areas of the skin. These immunohistochemical findings are supported by in vitro binding of Vn to keratin intermediate filaments. The reader is referred to a review article of K. Dahlbaeck for further discussion on the distribution in diseased skin (Dahlbaeck, 1993).

Another example for the localization of Vn in normal tissues is the skeletal system. Adhesive events are of critical importance for bone function (Lakkakorpi et al., 1991; Rodan, 1992; Young et al., 1992). For example, the initial adhesive interaction of the osteoclast with adhesive glycoproteins on the bone surface is required to initiate bone resorption. The physiological ligand(s) in the bone matrix has not yet been identified. Immunohistochemical staining reveal that Vn is present throughout the mineralized bone matrix of cancellous and cortical murine bone, whereas the cartilage is devoid of Vn staining (Seiffert, 1996). Similar results have been reported for human bone tissue (Grzesik and Robey, 1994). The Vn immunoreactivity does not appear to be derived from remaining blood present in blood vessels, since fibrinogen immunoreactivity is not present in the mineralized bone matrix. In addition, hydroxyapatite affinity chromatography raises the possibility that mineral interactions, at least in part, mediates the incorporation of Vn into the bone matrix (Seiffert, 1996). This study indicate that Vn is a specific component of bone tissue and raise the possibility that Vn is involved in the regulation of bone metabolism. It should be noted that Vn is associated with mineralization areas within the elastic fibers of pseudoxanthoma elasticum dermis (Contri et al., 1996), supporting that Vn interacts with mineralized fibers.

Vitronectin appears to be absent from the basement membrane of most tissues studied. However, Vn has been detected in diseased renal tubular basement membranes (Falk et al., 1987). A strong correlation between the deposition of the membrane attack complex of complement and Vn is found in the kidney of patients with glomerulonephritis, arteriosclerosis, and systemic lupus erythematosus (Falk et al., 1987; Bariety et al., 1989; French et al., 1992; Okada et al., 1993; Ogawa et al., 1994). These observations suggest that Vn may function to regulate immune reactions in the diseased kidney.

A common pattern of Vn staining can be observed in areas of sclerosis and necrosis in a number of tissues. For example, light microscopic studies of the liver reveal Vn deposition in areas of focal necrosis and in the portal tracts in patients with acute and chronic viral hepatitis, and in areas of fibrous deposition in the liver of patients with cirrhosis (Garred et al., 1993; Kobayashi et al., 1994). Vn deposition has also been reported in areas of fibrosis and necrosis of a number of different tissues, including the skin (for review, see Dahlbaeck, 1993), kidney (Ogawa et al., 1994), nerve tissue of patients with peripheral neuropathy and degenerative central nervous system disorders (Zanusso et al., 1992; Eikelenboom et al., 1994; Zhan et al., 1994), fibrotic and sclerotic lymphoid tissue (Reily and Nash, 1988; Castanos-Velez et al., 1995), germinal center areas in follicular lymphoid hyperplasia (Halstensen et al., 1988), fibrillar deposits in the connective tissue matrix around all types of breast cancer (Loridon-Rosa et al., 1988; Niculescu et al., 1992), myelofibrotic bone marrow (Reily and Nash, 1988), and in areas of acute myocardial and kidney infarction (Vakeva et al., 1993, 1995). Similar staining pattern are noted in intimal thickenings and fibrous plaques of atherosclerotic arteries in association with collagen bundles, elastic fibers, and cell debris in the vicinity of elastin (Niculescu et al., 1989; Guettier et al., 1989). Moreover, a monoclonal antibody that specifically stains rabbit atherosclerotic lesions has been shown to detect Vn (Sato et al., 1990). However, Vn is also detected in apparently normal organ-specific vessels of the uterus (D'Cruz and Wild, 1992), spleen (Liakka and Autio-Harmainen, 1992), gut (Halstensen et al., 1989), and kidney (French et al., 1992), suggesting that Vn accumulation in the vessel wall is not a specific marker for vascular disease. Moreover, we recently observed Vn immunoreactivity associated with the lamina elastica interna and externa of apparently normal human arteries and veins (Loskutoff, Seiffert, van Aken, unpublished observation). In general, Vn mRNA is not detectable by in situ hybridization in both normal and atherosclerotic blood vessels (unpublished observation). However, Sawa et al. (1993) provided evidence for Vn biosynthesis by neointimal cells in hepercholesterolemic rabbits. Thus, it remains unclear whether vascular depositions of Vn are derived from low levels of local biosynthesis or are plasma-derived.

The demonstration of Vn antigen in both normal and diseased tissues by immunohistochemistry combined with the absence of detectable Vn biosynthesis by some of these tissues (e.g., most of the vessel wall) suggest that specific mechanism are present that mediate the uptake of Vn into tissues. In vitro experiments have established some of the putative mechanism how Vn could reach extravascular sites (Voelker et al., 1993; reviewed in De Boer et al., 1995; Preissner and Poetsch, 1995). Multimeric Vn and Vn containing complexes interact with endothelial cell monolayers (de Boer et al., 1992). In an energy-dependent process, multimeric Vn is translocated either into lysosomal compartments of endothelial cells or transcytosed to the basolateral aspect in association with the basement membrane (Preissner and Poetsch, 1995). Alternatively, in areas of disturbed endothelial cell lining of the vessel wall in areas of tissue injury and necrosis, Vn might directly interact with extracellular matrix molecules.

A number of molecules may be Vn binding molecules in the extracellular matrix. Although Vn purified under denaturing conditions binds to type I-VI collagen, plasm Vn does not bind well to any collagen (Gebb et al., 1986; Izumi et al., 1988). Proteoglycans represent a second group of Vn binding structures in the extracellular matrix, since Vn interacts with heparin, heparan sulfate, fucoidan, and dextran sulfate (Barnes et al., 1985; Hayashi et al., 1985; Akama et al., 1986; Tomasini and Mosher, 1988). These data raise the possibility that glycosaminoglycans coupled to their core protein may bind Vn in vivo. Again, the plasma form of Vn shows little binding to heparin (Tomasini and Mosher, 1988; Izumi et al., 1989; Seiffert and Schleef,

1996). This suggests that multimerization and conformational changes in the molecule are required for the interaction with collagen and glycosaminoglycans. These multimers may either be generated upon ligand binding (e.g., thrombin/antithrombin III complexes [Tomasini and Mosher, 1988] or type 1 plasminogen activator inhibitor [Seiffert and Loskutoff, 1996]) or released from platelets in areas of tissue injury (Preissner et al., 1989; Seiffert and Schleef, 1996). Although several immunohistochemical studies suggest that Vn may be associated with elastin (see above), direct binding studies to substantiate these findings have not yet been reported. Vn is a substrate for tissue transglutaminase and coagulation factor XIIIa (Sane et al., 1990, 1991), raising the possibility that Vn may be covalently associated with yet unidentified extracellular structures. We recently reported that Vn associates with vimentin-type intermediate filaments in vivo, in cell culture, and in a purified protein system (Podor et al., 1992). These filaments are expected to be exposed upon tissue injury and necrosis. Again, little binding of native Vn to intermediate filaments were observed. Vn also binds to keratin filament aggregates after incubation of tissue section with purified denatured Vn (Hintner et al., 1989). Thus, binding of Vn to intermediate filaments in both normal and injured tissues may provide a mechanism for its incorporation into tissues. It is apparent that many of the incorporation and binding mechanism into tissues require the multimeric form of Vn. The conversion of the native form of Vn to the multimeric, conformationally altered form is believed to be irreversible. As a result, the tissue form of Vn is expected to be present in the multimeric form. Although this concept is intriguing, further experimental evidence is required to support this hypothesis.

The functions of tissue Vn are similarly unclear. As a minimum, Vn depositions can be viewed as a marker of tissue injury and necrosis and may not be causally related to the development/progression of the underlying disease. This conclusion however appears unlikely, since patients with Vn deficiencies have not been observed and Vn-like molecules are highly conserved during phylogeny and are even detected in plants (Wagner et al., 1992; Hayashi, 1993). Wound healing requires a spatial and temporal regulation of adhesive interactions and proteolytic enzyme cascades. In addition, systemic injury which could question the existence of the whole organism need to be prevented. As a first step, Vn may serve to localize injury processes by localizing and concentrating type 1 plasminogen activator inhibitor in areas of injury, resulting in a local fibrinolytic shutdown. Similarly, blocking of glycosaminoglycan stimulated inhibition of procoagulatory enzyme will result in a local prothrombotic state. As a result, the injury process will be localized and systemic injury processes prevented. While such a process is expected to be beneficial in acute injury, repetive, chronic injury, as observed for example in rheumatoid arthritis, lung fibrosis, and atherosclerosis, will result in an extensive accumulation of Vn into the thickened extracellular matrix, thereby contributing to the pathophysiology of the disease process.

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