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Vascular Adhesion Protein-1 and Hepatocellular Cancer

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1. Introduction

The extravasation of leukocytes from the vasculature to the tissue space is a fundamental response of the normal immune system. A multistep model of leukocyte adhesion to vascular endothelium has been characterized, although details of the signaling differ between tissues [1-9].

Lymphocytes recirculate between blood and tissues as part of the immune surveillance process, and recent evidence suggests that specialized subsets of T cells exhibit discrete tissue-specific patterns of recirculation in vivo [2, 10].

These cells are directed to particular tissues by combinations of adhesion molecules and chemokines that control the lymphocyte recognition of and adhesion to endothelium. For example, memory T cells that recirculate to the gut lamina propria express integrin $\alpha_4\beta_7$ and bind to an endothelial ligand, namely, mucosal cell adhesion molecule-1, which is detected in gut endothelium [11]. Conversely, T cells that migrate to the skin do not express $\alpha_4\beta_7$ but do express high levels of the lymphocyte Ag, Which binds to E-selectin on dermal endothelium [12] .The liver is a major site of Ag exposure and contains large numbers of lymphocytes even under normal conditions. These lymphocytes are differentiated lymphocytes that are displaced by apoptosis [13-17]. It is thus likely that tissue-specific signals regulate lymphocyte recruitment to the liver [18].

The hepatic cells are formed into ranks by specialized endothelium that supports lymphocyte adhesion and recruitment in a low-shear environment [19]. Hepatic endothelium has a discrete phenotype compared with endothelium from other vascular beds.

Several cell adhesion molecules play an important role in this complex process and stabilize the adhesion and diapedesis of leukocytes across the endothelial barrier, similar to the manner in which human hepatic endothelium cells secrete the endothelial adhesion molecule vascular adhesion protein-1 (VAP-1)[-9].

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The VAP-1 molecule is a 170 kDa homodimeric glycoprotein that consists of two 90 kDa subunits that are held together by disulfide bonds [20]. VAP-1 has a large extracellular domain, a single-pass transmembrane domain, and a short cytoplasmic cue [21]. The molecule has ample sialic acid moieties that are required for its adhesive function, which was shown based on the inability of VAP-1 to mediate lymphocyte adhesion to desialylated vessels [20] . The leukocyte ligand for VAP-1 is presently unknown.

Serum measurements of VAP-1 are performed by enzyme-linked immunosorbent assay[22].

A possible role for VAP-1 was shown in adhesion assays. VAP-1 may have a normal physiological role in maintaining the proadhesive status of PBLs toward endothelia since its concentration in normal individuals is within the range that is used in these adhesion experiments.

Some reports have shown that significantly elevated levels of VAP-1 were found in patients with liver diseases [22, 23]. Patients had increased systemic circulation, and VAP-1 levels in these patients were higher, suggesting that VAP-1 could engender biological effects. It is interesting that patients with hepatocellular carcinoma had higher VAP-1 values than patients with inflammatory liver diseases, which agrees well with the finding of high VAP-1 expression in primary liver tumors but not in liver metastases [22-24]. Other non-hepatic inflammatory diseases were not related to increased levels of VAP-1 [23]. An increase in VAP-1 levels is specific to particular inflammatory diseases. The specificity of increased VAP-1 levels for certain liver diseases (hepatocellular carcinoma) justifies further characterization of the role of VAP-1 in the inflammatory activity of these diseases.

In conclusion, VAP-1 is present in the serum of healthy individuals at a level similar to those of most other soluble endothelial adhesion molecules [25]. The concentration of VAP-1 is higher in certain liver diseases than the levels of other known circulating endothelial adhesion molecules [23, 25].

2. Vascular adhesion protein-1 and hepatocellular cancer

Unmixed lymphocytes travel continuously throughout the body in search of antigens. Blood-borne lymphocytes leave the circulation by binding to the endothelium of specialized postcapillary high endothelial venules (HEVs) in lymph nodes, move through the tissue stroma, and later return to the circulation via efferent lypmhatics [26]. Elemental lymphocytes can freely circulate through both peripheral lymph nodes (PLNs) and mucosa-associated lymphatic tissues, which represent two functionally different re-circulatory systems. It has been known for some time that T cells immigrate and bind to PLN 2-5 times more frequently than B cells. On the contrary, B cells adhere 2-3 times more frequently than T cells to HEVs in mucosal lymphatic organs. When unmixed lymphocytes adhere to their related antigens, the migratory properties of the activated lymphocytes change dramatically. They no longer freely circulate through different lymphatic organs but instead selectively extravasate at sites of the original antigenic and in related lymphoid tissues [27].

Lymphocyte exchange between the circulation and tissues is essential for the appropriate function of the immune system. In the extravasation function, blood-borne cells make primary contacts with endothelial adhesion molecules, which may stimulate grappling, activation, entrenched binding, and finally transmigration [1, 27]. The grappling cells can be

exposed to activating stimuli such as chemokines that can reinforce the integrin-dependent adhesion of leukocytes and migration through vessel walls using adhesion molecules from immunoglobulin and other super-families as well as local protease activity. Immune function is predicated on the continuous exchange of lymphocytes between the blood and tissue and is regulated by molecular interactions between the circulating lymphocytes and ligands on the surface of endothelial cells [28].

VAP-1 was described after monoclonal antibody (mAB) 1B2 immunoprecipitation, which gave a 170- to 180-kDa homodimeric sialoglycoprotein that was formed by two 90 kDa subunits, bound by disulfide bonds, with close sequence homology to the copper-dependent semicarbazide-sensitive amine oxidases (SSAO) [1, 20]. Both the transmembrane and soluble forms of VAP-1 exhibit monoamine oxidase activity [29]. A rabbit homolog of VAP-1 has been shown to function under in vivo conditions during the primary temporary interactions between endothelial and lymphoid cells [20]. VAP-1 has a large extracellular domain, a single-pass transmembrane domain, and a short cytoplasmic cauda [20].

Soluble adhesion molecules may have definite and adverse physiological effects. They may function as inhibitors of cell to cell adhesion by competing with their membrane-bound forms.

Serum measurements of VAP-1 were performed by enzyme-linked immunosorbent assay (ELISA). Blood was drawn into serum tubes and allowed to clot for at least 30 min, centrifuged at 3000 rpm for 10 min and kept frozen at -70°C [22]. Wells of microtiter plates were coated with 100 µl of the anti–VAP-1 mAb TK8-18 at 10 µg/ml in 0.1 M $NaHCO_3$ buffer (pH 9.6), stored at 4^0Covernight, and then kept at 37°C for 1 h. The wells were washed 6 times with 0.1% Tween 20 in phosphate buffered saline (PBS) and then blocked by the addition of 200 µl of PBS containing 1% gelatin and 1% nonfat milk powder (blocking solution) for 45 min at room temperature to prevent nonspecific adsorption. After washing the wells 6 times with Tween/PBS, 175 µl of each serum sample was added to the wells, and the plates were left at room temperature for 1 h. The wells were then washed six times with Tween/PBS and incubated with 100 µl of the biotinylated anti–VAP-1 mAbTK8-14 or biotinylated control mAb Hermes-3 at room temperature for 1 h. After six washes with Tween/PBS, 100 µl of streptavidin–horseradish peroxidase was added to the wells, and the plates were allowed to incubate at room temperature again for 1 h. Thereafter, the plates were washed six times with Tween/PBS and finally developed with a chemoluminescence ELISA reagent (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions. All serum samples were measured at 1:25 dilution. Each sample was measured in triplicate, and the anti–VAP-1 value was calculated by subtracting the mean background value of the negative control from the mean value of VAP-1 [23].

Each assay included a titration of the quantified tonsil lysate; this titration was used to generate a standard curve. To obtain a protein milieu similar to the serum samples, tonsil lysate standards were diluted in blocking solution that contained as much VAP-1–depleted human serum as the test samples contained patient serum. Calculations of the amount of sVAP-1 in the serum samples were made by comparing the specific VAP-1 values with a standard curve of titrated tonsil lysate using a linear regression analysis [23].

The adhesive function of VAP-1 is important in optimal oligosaccharide modifications since desialylated VAP-1 does not bind to lymphocytes [30]. The lymphocyte ligand of VAP-1 is

unknown whereas the enzymatic reaction takes place independently within the rotund buried catalytic center of the molecule [31].

VAP-1 is postulated as a mediator of adhesion of effector lymphocytes to tumor vasculature. It is an endothelial adhesion molecule that is up-regulated at inflammation sites and mediates lymphocyte binding to inflamed endothelium [23].

Similar to the membrane-bound endothelial VAP-1 [1], circulating VAP-1 involves effusive sialic acid decorations (moieties). The finding of effusive sialic acid remnants in VAP-1 is especially noteworthy because the adhesive function of VAP-1 depends on its sialic acids [1]. VAP-1 is a deeply sialylated molecule that might be functionally active.

The probable biological effects of VAP-1 could affect the expression or function of other adhesion molecules in vivo or in vitro in primary endothelial cells and in liver endothelial cells. These effects of VAP-1 on the synthesis of other adhesion molecules such as fucosyltransferases or sulfotransferases might contribute to the significantly rising capability of VAP-1 transfectants to effect lymphocyte accession.

VAP-1 mediates the leukocyte subtype-specific recognition of HEVs under nonstatic conditions in humans, and hence, it represents a previously unknown method of achieving selectivity of the leukocyte-endothelial interactions in the multistep adhesion cascade. VAP-1 is able to mediate the adhesion of PLN and HEV. Moreover, VAP-1 extends the role of carbohydrate-dependent lymphocyte–endothelial cell interactions [20].

VAP-1 mediates HEV binding of lymphocytes but not of monocytes. Adhesion of T and B cells was independent of VAP-1. VAP-1 mediated oligosaccharide-dependent adhesion to endothelial cells under non-static harvest conditions [20], implying that it is involved in the adhesion cascade.

Hepatic endothelial cells are lined by specialized endothelium that supports lymphocyte adhesion and recruitment in an optimal low-shear environment [31]. Hepatic endothelium has a phenotype that is different from the endothelium of other vascular beds. In vivo hepatic endothelial cells express low levels of CD31 which are the most expansive entrapment receptors in other tissues [32]. Human hepatic endothelial cells secrete the endothelial adhesion molecule VAP-1 [33,] which is major defective from non-inflamed vessels in extralymphoid organs [34]. The ability of VAP-1 to mediate sialic acid-dependent adhesion suggests that it could have a particular function in the liver by mediating shear-dependent adhesion in the selectins [15]. However, human liver endothelial cell behavior under conditions of shear stress is unknown.

VAP-1 on liver endothelium promotes lymphocyte adhesion under laminar shear stress but not in hepatic roaming in vivo [35]; VAP-1 is a mediator of lymphocyte transendothelial migration, but the capability of VAP-1 to promote adhesion and transendothelial migration is blocked by specific inhibitors of its enzyme activity [35]. Functional characterization of VAP-1 has not been conducted because the molecule is not expressed on the cell surface of human endothelial cell lines [22].

In some studies, rising levels of serum VAP-1 were found in patients with hepatocellular cancer [36, 37]. These results suggest that increasing levels of VAP-1 contribute to the elevated

adhesion of lymphocytes to vascular endothelial cells on the periphery of primary liver tumors. This mechanism could increase the immune response at the tumor where other adhesion molecules involved in the multistep adhesion cascade are also elevated in primary liver tumors. The rise in VAP-1 expression is specific to liver tumors because other non-hepatic tumors are not associated with elevated levels of VAP-1.

VAP-1 expression occurs in the hepatic vascular bed [38, 39]. This occurrence suggests that some of the elements of the adhesion cascade in the hepatic vascular bed differ from those in post capillary venules.

VAP-1 mediated adhesion is consistent with the finding of VAP-1–dependent lymphocyte adhesion to rat peripheral lymph node endothelial cells transfected with human VAP-1 and with recent intravital studies where it has been shown to act as a brake for neutrophils in rabbit mesenteric blood vessels [39]. The precise nature of the adhesion mediated by VAP-1 was unclear, although the total number of adherent cells was reduced with the inhibition of VAP-1, suggesting that its receptor is active at different points in the adhesion cascade.

The inhibition of adhesion by blockade of VAP-1 had a marked inhibitory effect on lymphocyte transendothelial migration that was independent of its capability to assist adhesion. This effect was specific for VAP-1. It has also been proposed that VAP-1 mediates the transmigration of adherent leukocytes [28].

VAP-1 is a monoamine oxidase, and this functionality was demonstrated with soluble VAP-1 protein [40]. Recent reports suggest that the adhesive and enzymatic functions of VAP-1 are closely linked. Some specific inhibitors block the enzymatic activity of VAP-1, and the specific semicarbazide-sensitive amine oxidase inhibitor (SSAO), semicarbazide, and a broad-acting monoamine oxidase inhibitor, hydroxylamine, both decreased lymphocyte adhesion and transmigration. The enzymatic activity of VAP-1 is thought to be important in the adhesion of lymphocytes to VAP-1–transfected cells.

This effect was specific for hepatic endothelial cells that express VAP-1 since adhesion and transmigration of lymphocytes was observed. Other molecules have been shown to share adhesive and enzymatic properties, but VAP-1 is the only adhesion molecule with amino oxidase activity [23, 40]. The active site of the SSAO in VAP-1 may mediate adhesion via interactions with immobilized amine residues on the lymphocyte surfaces [40]. The fact that the inhibitors had a similar effect on transendothelial migration is the first demonstration of enzymatic regulation of this process.

The identification of VAP-1 as a new contact-initiating ligand suggests that it may be part of an optimal molecular pathway for regulating the specificity and multiplicity of the initial steps of lymphocyte–endothelial cell interactions.

VAP-1 may be a molecule that is template expressed in the liver tumor vasculature but down-regulated in some tumors during the growth of the malignancy or not up-regulated in some other tumors. Since VAP-1 clearly binds to the blood vessels of liver tumors, it is conceivable that the up-regulation of VAP-1 in liver tumors with a low level of VAP-1 expression may improve the treatment of hepatocellular carcinoma [20, 40, 41].

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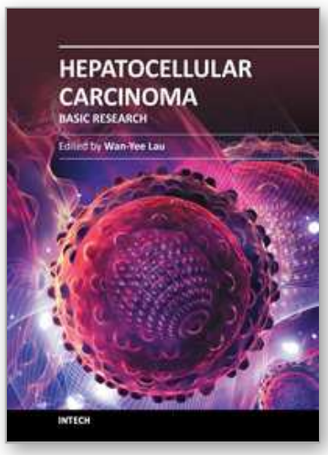

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Hepatocellular Carcinoma represents a leading cause of cancer death and a major health problem in developing countries where hepatitis B infection is prevalent. It has also become increasingly important with the increase in hepatitis C infection in developed countries. Knowledge of hepatocellular carcinoma has progressed rapidly. This book is a compendium of papers written by experts to present the most up-to-date knowledge on hepatocellular carcinoma. This book deals mainly with the basic research aspect of hepatocellular carcinoma. The book is divided into three sections: (I) Biomarkers / Therapeutic Target; (II) Carcinogenesis / Invasion / Metastasis; and (III) Detection / Prevention / Prevalence. There are 18 chapters in this book. This book is an important contribution to the basic research of hepatocellular carcinoma. The intended readers of this book are scientists and clinicians who are interested in research on hepatocellular carcinoma. Epidemiologists, pathologists, hospital administrators and drug manufacturers will also find this book useful.

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