
UNIVERSITE DE LAUSANNE
FACULTE DE BIOLOGIE ET DE MEDECINE

Institut de Pathologie
Directeur : Professeur Frederik T. Bosman

Immunohistochemical Expression of Endothelial Markers CD31, CD34,
von Willebrand Factor and Fli-1 in Normal Human Tissues.

THESE

présentée à la Faculté de biologie et de médecine de l'Université de Lausanne
pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

Marc Pusztaszeri

BMTE 3380

QW
573
Pus

Médecin diplômé de la Confédération Suisse
Originaire de Jouxens-Mézery

Lausanne
2006

Résumé du travail de thèse

Introduction : Les différentes cellules endothéliales du lit vasculaire ont de nombreuses similitudes fonctionnelles et morphologiques. Cependant, elles présentent également une importante hétérogénéité structurelle et fonctionnelle qui peut avoir des implications notamment dans l'angiogenèse et le développement des maladies cardio-vasculaires. Peu d'études ont été publiées au sujet de l'expression et de la distribution des marqueurs endothéliaux dans les tissus humain normaux.

Objectif : Nous avons étudié l'expression immunohistochimique des marqueurs endothéliaux CD31, CD34, vWF et Fli-1 dans les vaisseaux périphériques du rein, du poumon, de la rate, du foie, du cœur et des gros vaisseaux ; incluant l'aorte, la veine cave inférieure, l'artère rénale ainsi que les artères et veines pulmonaires et fémorales.

Matériel et méthodes : Les échantillons tissulaires ont été obtenus à partir de matériel d'autopsie et de biopsies. Le matériel a été fixé en formaline et inclus en paraffine. Les coupes de paraffine ont été colorées immunohistochimiquement avec CD31, CD34 et vWF. Les biopsies ont également été colorées immunohistochimiquement avec Fli-1, D2-40 et Lyve-1.

Résultats : L'expression immunohistochimique de ces marqueurs est hétérogène dans les différents organes étudiés. Dans le rein, l'endothélium fenêtré des glomérules exprime fortement CD31 et CD34. Par contre, il n'exprime pas ou alors de manière faible et focale vWF. Dans le poumon, les capillaires alvéolaires expriment fortement CD31 et CD34 mais sont habituellement négatifs pour le vWF. L'expression de vWF augmente graduellement avec le calibre vasculaire dans le poumon. Les sinusoides de la rate expriment CD31 de manière diffuse mais ils n'expriment pas CD34. Les sinusoides du foie expriment CD31 de part et d'autre des lobules. Par contre, CD34 est exprimé seulement dans la région périportale. L'expression de Fli-1 dans les cellules endothéliales est ubiquitaire et ne varie pas suivant le type de vaisseau ou d'organe. Fli-1 est également exprimé dans d'autres types de cellules, essentiellement des lymphocytes. D2-40 est exprimé seulement dans l'endothélium des vaisseaux lymphatiques. L'expression de Lyve-1 dans ce matériel de routine était inconstante et non reproductible.

Conclusion : Ces résultats indiquent que l'expression des marqueurs endothéliaux CD31, CD34 et vWF est hétérogène dans le lit vasculaire et qu'elle varie entre différents vaisseaux et différents compartiments anatomiques du même organe. D2-40 ne marque que les cellules endothéliales lymphatiques.

ARTICLE

Immunohistochemical Expression of Endothelial Markers CD31, CD34, von Willebrand Factor, and Fli-1 in Normal Human Tissues

Marc P. Pusztaszeri, Walter Seelentag, and Fred T. Bosman

Institute of Pathology, Lausanne, Switzerland

SUMMARY Few systematic studies have been published comparing the expression and distribution of endothelial cell (EC) markers in different vascular beds in normal human tissues. We investigated by immunohistochemistry the expression of CD31, CD34, von Willebrand factor (vWF), and Fli-1 in EC of the major organs and large vessels. Tissue samples obtained from autopsies and biopsy specimens were routinely processed and stained immunohistochemically for CD31, CD34, and vWF. Biopsy material was also stained immunohistochemically for Fli-1, D2-40, and Lyve-1. The expression pattern of the markers was heterogeneous in some of the organs studied. In the kidney, fenestrated endothelium of the glomeruli strongly expressed CD31 and CD34 but was only focally positive or completely negative for vWF. Alveolar wall capillaries of the lung strongly stained for CD31 and CD34 but were usually negative for vWF. The staining intensity for vWF increased gradually with the vessel caliber in the lung. Sinusoids of the spleen and liver were diffusely positive for CD31. They were negative for CD34 in the spleen and only expressed CD34 in the periportal area in the liver. Fli-1 was expressed in all types of EC but also in lymphocytes. D2-40 stained lymphatic endothelium only. Lyve-1 immunostaining was too variable to be applied to routinely processed tissues. The expression of EC markers CD31, CD34, and vWF in the vascular tree is heterogeneous with a specific pattern for individual vessel types and different anatomic compartments of the same organ. D2-40 labels lymphatic EC only. (*J Histochem Cytochem* 54:385–395 2006)

KEY WORDS

platelet endothelial cell
adhesion molecule 1
CD34
von Willebrand factor
Fli-1
endothelial cells
heterogeneity
microvasculature

ENDOTHELIAL CELLS (EC) have many functions and play a central role in the control of coagulation, thrombolysis, vascular tone, permeability, inflammation, tissue repair, and angiogenesis. They constitute a heterogeneous cell population in the human body. Functions and, as a consequence, molecular characteristics of EC vary along the vascular tree and in the same organ between different vessels (Cines et al. 1998; Müller et al. 2002a). Few studies have been published with the intention to systematically compare the expression and distribution of EC markers in different vascular beds in normal human tissues in vivo. Hence, scarce data are available on the immunohistochemical expression pattern of commonly used EC markers like CD31, CD34, and von

Willebrand factor (vWF) on normal endothelium. This is also the case for more recent EC markers used in vascular tumors, such as Fli-1, and for lymphatic endothelial markers such as Lyve-1 or D2-40.

The few studies published have mostly been restricted to the lung. Müller et al. (2002a) showed that CD31 is usually strongly and homogeneously expressed by all human pulmonary EC. The expression pattern of CD34 and vWF was found to be more heterogeneous. The strongest expression of CD34 was found in EC of capillaries, followed by arteries, veins, arterioles, and venules. Two studies have been performed comparing the expression of vWF in human pulmonary EC (Kawanami et al. 2000; Müller et al. 2002b). Also in these studies, CD31 showed homogeneous intense staining in all pulmonary EC. In contrast, vWF-specific antibody yielded negative or weakly positive staining reactions in capillary EC. The staining intensity increased with the vessel caliber. Veins exhibited the

Correspondence to: Marc P. Pusztaszeri, Institute of Pathology, Rue du Bugnon 25 1011, Lausanne, Switzerland. E-mail: Marc.Pusztaszeri@chuv.ch

Received for publication August 31, 2004; accepted September 28, 2005 [DOI: 10.1369/jhc.4A6514.2005].

Table 1 Antibodies used in the study

Antibody	Dilution	Source	Clone	Incubation
Monoclonal CD31	1/40	DakoCytomation; Glostrup, Denmark	JC70A	Overnight
Monoclonal CD34	1/16	Immunotech; Marseilles, France	Qbend 10	40 min
Polyclonal factor 8	1/20	Novocastra; Newcastle, UK	ED15'	40 min
Polyclonal Fli-1 (C19)	1/20	Santa Cruz Biotechnology; Santa Cruz, CA	sc-356	40 min
Monoclonal D2-40	1/400	Signet; Dedham, MA	730-01	40 min
Lyve-1	1/20	Reliatech; Braunschweig, Germany	102-PA50	Overnight

The 4- μ m paraffin sections were deparaffinized in xylol and rehydrated in graded alcohol series. Endogenous peroxidase was inhibited using 3% H₂O₂ in methanol. The sections were then washed in distilled water and heated in a microwave oven (in citrate buffer 10 mM, pH 6, for CD31, CD34, Fli-1, and Lyve 1 and EDTA 1 mM, pH 7.5, for factor VIII) 15 min for epitope retrieval. No pretreatment was needed for D2-40. Slides were incubated first with normal horse serum (1/30 avidin 10%) for CD31 and CD34 and with normal goat serum (10% avidin) for factor VIII and Fli-1 for 5 min and then in biotin for 10 min. Endogenous biotin was inhibited with a Vector Blocking kit (Vector Laboratories; Burlingame, CA). The slides were then incubated at 20C for 40 min with monoclonal antibodies for CD31, CD34, and D2-40 and polyclonal antibody for factor VIII and Fli-1. For CD31 and Lyve-1, the slides were incubated overnight at 8C. The slides were incubated with anti-mouse/rabbit biotinylated bridging antibodies (dilution 1/200) for 30 min. Sections were then washed and incubated with standard avidin-biotin complex (ABC; DakoCytomation, Glostrup, Denmark) for 30 min. Antibody binding was revealed using H₂O₂ as a substrate and diaminobenzidine as chromogen. Counterstaining was performed with hematoxylin.

Results

The expression pattern for CD31, CD34, vWF, and Fli-1 in the different tissues we studied was strikingly heterogeneous. The differences concerned especially the small vascular beds including continuous and fenestrated capillaries and sinusoids. Major differences were seen in the parenchymatous organs and are summarized in Table 2. There were no significant differences between the autopsy and the biopsy tissues.

Kidney

The fenestrated endothelium of the glomeruli always strongly expressed CD31 and CD34 but was only focally positive or completely negative for vWF (Figures 1A-1C). In one biopsy case, the glomeruli were diffusely positive for vWF. In the three autopsy cases from hypertensive patients, the glomeruli were weakly or moderately positive for vWF as compared with the autopsy cases from patients without hypertension. More interstitial capillaries stained for CD34 than for CD31 or vWF. Fli-1 usually stained the nuclei of EC in the glomeruli (Figure 1D), making it easier to differentiate between EC and other types of cells also present in the glomeruli, such as podocytes or mesangial cells.

In addition, EC of arterioles, venules, and interstitial capillaries were stained. There were, however, some cases in which the interpretation was difficult as a result of important background staining.

Lungs

Capillaries in the alveolar wall strongly stained for CD31 and CD34 but were usually negative for vWF (Figures 2A-2C). The staining intensity for vWF also increased gradually with the vessel caliber. Small arterioles and venules showed light staining but larger arteries and veins showed intense staining. The staining intensity did not vary according to vessel type or caliber for CD31 and CD34. Alveolar macrophages were positive for CD31 with a membranous pattern of staining but were negative for CD34 and vWF. Fli-1 diffusely stained the nuclei of EC of the different vessel types in the lungs

Table 2 Staining of endothelial cells for CD31, CD34, vWF, and Fli-1 in various normal adult tissues

Tissue	CD31	CD34	VWF	Fli-1 (nuclear)
Kidney				
Glomeruli	+++	+++	0/+	+++
Capillaries	++	+++	++	+++
Venules	+++	+++	+++	+++
Arteries	+++	+++	+++	+++
Lung				
Capillaries	+++	+++	0/+	+++
Arterioles	+++	+++	++	+++
Venules	+++	+++	++	+++
Veins	+++	+++	+++	+++
Arteries	+++	+++	+++	+++
Liver				
Periportal sinusoids	+++	+++	++	+++
Centrolobular sinusoids	+++	0/+	++	+++
Centrolobular veins	+++	+++	++	+++
Portal venules	+++	+++	++	+++
Arterioles	+++	+++	++	+++
Spleen				
Sinusoids	++	0	++	++ ^a
Capillaries	0	+++	0/+	++ ^a
Central arteries	+++	+++	++	++ ^a
Venules	+++	+++	++	++ ^a
Lymph nodes				
Capillaries	+++	+++	++	++ ^a
Marginal sinuses	+++	0/+	0/+	++ ^a
High endothelial venules	+++	+++	+++	++ ^a

Intensity of immunohistochemical staining: 0, absent; +, low; ++, medium; +++, high.

^aMost cells present (lymphocytes) are stained.

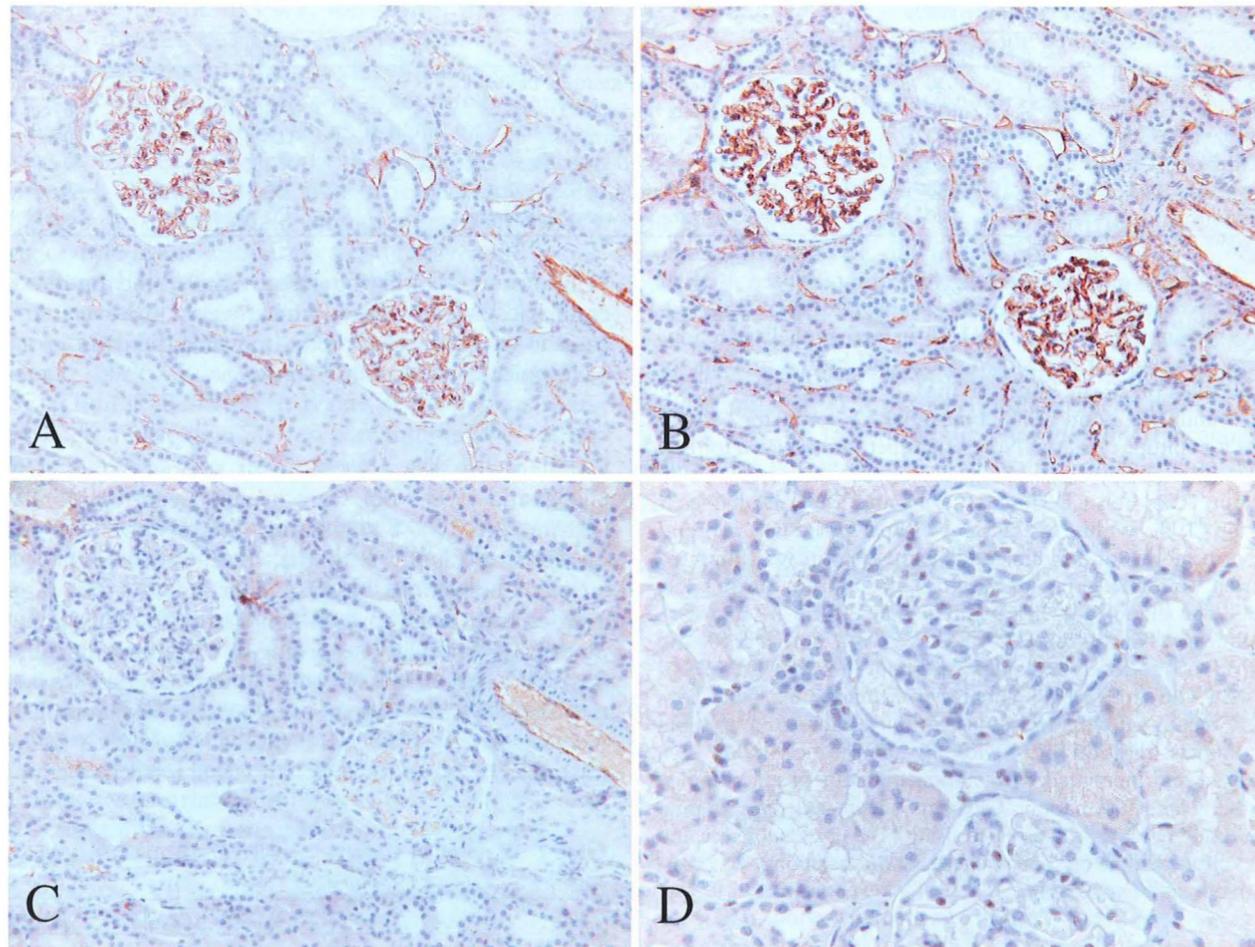


Figure 1 (A) CD31 expression in the kidney showing 2 glomeruli with strong positivity. (B) CD34 expression pattern in the kidney is similar to CD31 but more interstitial capillaries are stained. (C) In contrast to CD31 and CD34, the glomeruli are negative for von Willebrand factor (vWF). (D) Fli-1 nuclear immunostaining of EC present in the glomeruli and interstitial capillaries.

(Figure 2D). However, the nuclei of lymphocytes were also stained, as well as the cytoplasm of some alveolar wall cells probably corresponding to mastocytes.

Spleen

Sinusoidal EC were diffusely positive for CD31 but negative for CD34 (Figures 3A and 3B). In the red pulp, CD34 only marked capillaries.

vWF staining was similar to that for CD31 but slightly less intense (Figure 3C). There were no differences in the staining pattern of the central arteries, veins, and other vessels present in the spleen. Fli-1 stained the nuclei of EC present in the sinusoids but also the lymphocytes present in the white pulp. The larger vessels including the central arteries also stained (Figure 3D).

Liver

Sinusoidal EC continuously expressed CD31 from the portal space to the centrilobular vein (Figure 4A). In

contrast, CD34 was expressed only in the periportal area, the centrilobular sinusoids being mostly negative (Figure 4B). Expression of vWF was similar to that of CD31 (Figure 4C). Fli-1 usually stained the nuclei of EC present in the sinusoids, portal vein, centrilobular vein, and hepatic artery (Figure 4D). A granular cytoplasmic reactivity was also occasionally seen in hepatocytes.

Heart

The staining pattern of the intramyocardial capillaries was different between CD31 and CD34. More capillaries showed CD34 than CD31 immunoreactivity. The pattern of staining for vWF was similar to that for CD31. The endocardial endothelium strongly stained for CD31 but irregularly and less intensely for CD34 and vWF. Capillaries in fat tissue were positive for CD34. EC from interstitial capillaries, arterioles, and venules present in the myocardium also stained for Fli-1. A diffuse cytoplasmic background staining of cardiomyocytes was also seen with Fli-1.

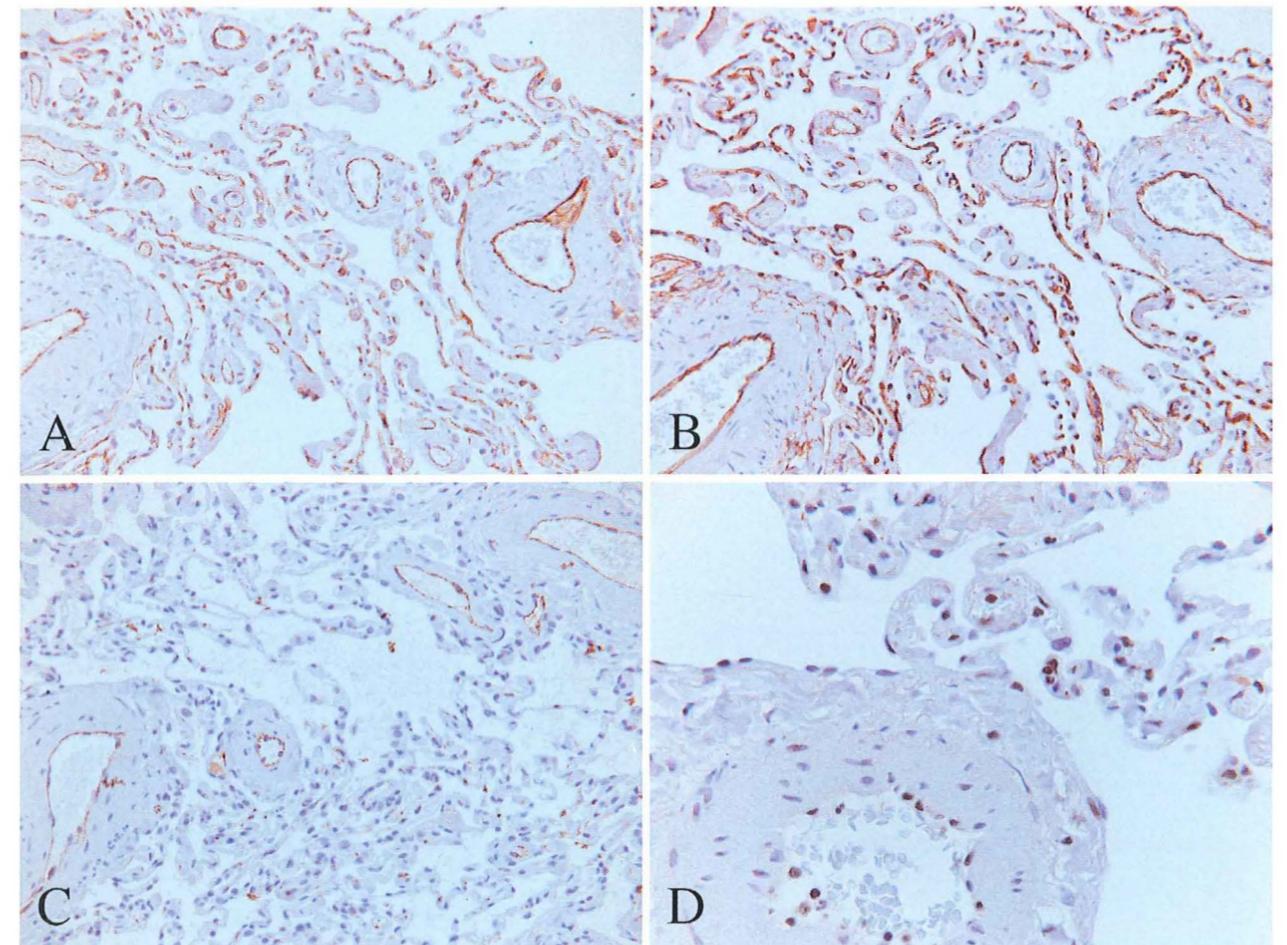


Figure 2 Alveolar capillaries and arterioles in the lung showing strong expression of CD31 (A) and CD34 (B). (C) In contrast, alveolar capillaries are negative for vWF, whereas arterioles and venules are positive for vWF. (D) Fli-1 nuclear immunostaining of endothelial cells (EC) from alveolar capillaries and arterioles.

Skin

Small arteries, arterioles, venules, and capillaries present in the skin were all stained with CD31, CD34, vWF, and Fli-1 in a similar fashion. Hair follicle cells were also occasionally positive for CD34. In the dermis, a reticular pattern of staining was seen with CD34, probably corresponding to CD34-positive mesenchymal cells (fibroblasts) and/or components of the extracellular matrix. Fli-1 also stained some lymphocytes present in the upper dermis and epidermis (granulation tissue).

Bone Marrow

Bone marrow sinuses, characterized by a discontinuous EC lining, were positive for CD31, CD34, and vWF. Capillaries, small arterioles, and venules present in the bone marrow in small amounts were also positive and did not show any significant differences for the above markers. Megakaryocytes were strongly positive for

CD31 and vWF but negative for CD34. Myeloid and erythroid lineage cells were usually negative for the above markers, some cells occasionally being positive for CD31. In some cases a slight background staining was seen, especially with vWF. Fli-1 immunostaining in the bone marrow did not show nuclear staining, presumably due to the decalcification process. Only in one or two cases, the nuclei of some megakaryocytes and of the myeloid lineage cells were positive.

Lymph Nodes

Sinusoidal EC of the marginal (subcapsular) and medullary sinuses were positive for CD31 but usually negative or focally and faintly positive for CD34 and vWF (Figures 5A–5C). In one case, sinusoidal EC of the marginal sinuses were positive for CD34 with the same intensity as for CD31. High endothelial venules were positive for CD31, CD34, and vWF. In the lymph node capsule, a reticular pattern of staining was also seen

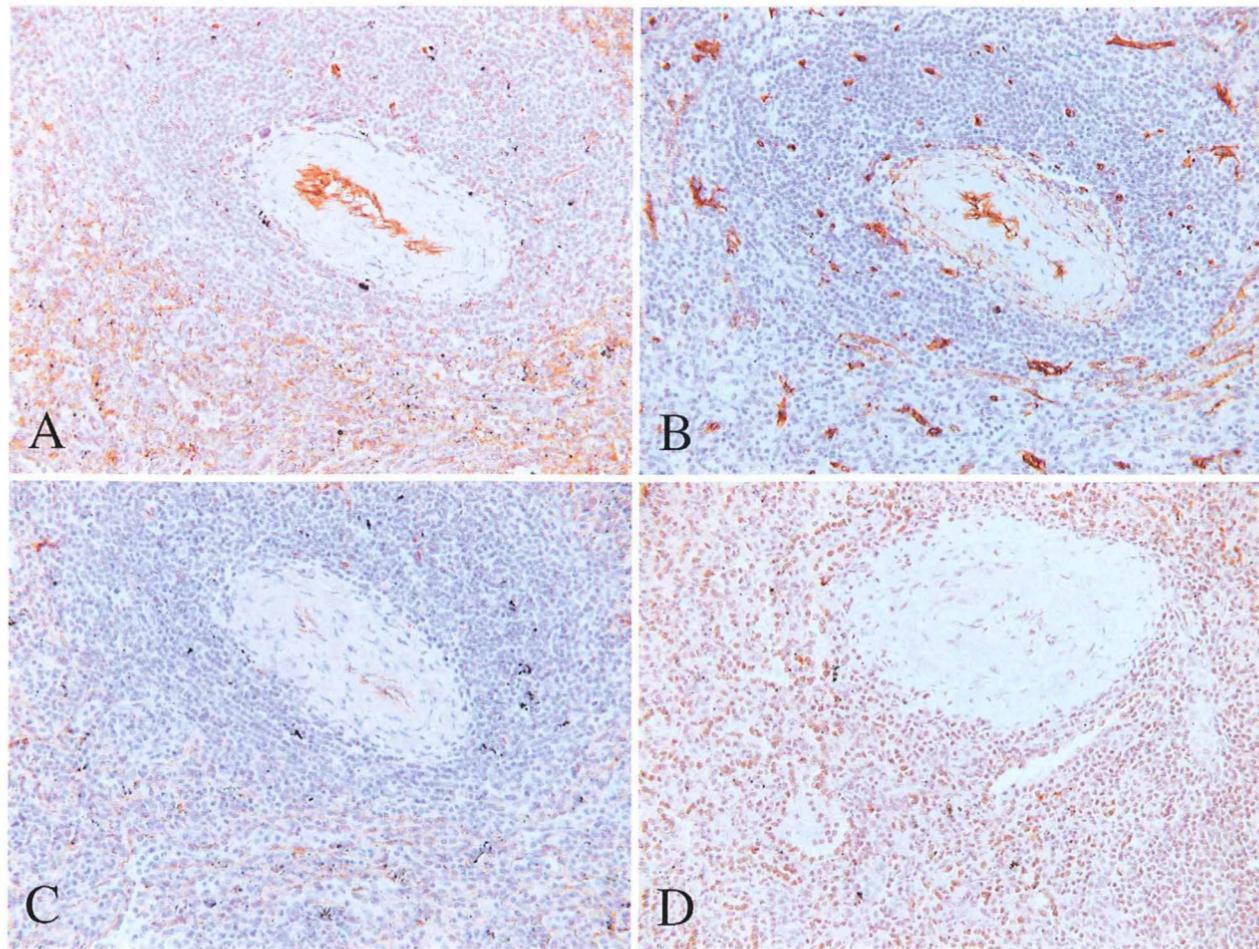


Figure 3 (A) CD31 expression in the spleen showing positive central arteries and sinusoids with a diffuse positivity. (B) CD34 expression in the spleen; in contrast to CD31, sinusoids are negative but interstitial capillaries stain for CD34. (C) The expression of vWF in the spleen is similar to CD31, but with less staining intensity. (D) Fli-1 immunostaining in the spleen; most cells present including EC are positive.

with CD34, probably corresponding to CD34-positive mesenchymal cells. Sinus histiocytes were positive for CD31 but negative for CD34 and vWF. Because Fli-1 stained most lymphocytes present in the lymph node, evaluation of EC staining was difficult, but EC at all levels seemed immunopositive for Fli-1 (Figure 5D).

Large Vessels

Large Arteries (Thoracic and Abdominal Aorta, Pulmonary Artery). The staining pattern of the endothelium was similar for CD31 and CD34 in the various large arteries we investigated. Due to loss of endothelial cell integrity, staining of the endothelium was often discontinuous for CD31 and CD34. This was not the case with vWF, which showed a continuous pattern of staining with the regions depleted of EC still being stained for vWF. CD34 also stained some components of the media, probably corresponding to smooth muscle cells.

Middle-sized Arteries (Renal Artery, Femoral Artery). Renal arteries taken from autopsy cases showed a continuous and strong staining for vWF. The staining pattern was usually discontinuous for CD31 and CD34, the intensity being less for vWF. CD34 also stained some components of the media, probably corresponding to smooth muscle cells.

Large Veins (Inferior Vena Cava, Pulmonary Vein) and Middle-sized Veins (Femoral Vein). The staining pattern of the endothelium was similar for CD31, CD34, and vWF for the different veins investigated.

Lymphatic Endothelium

In all parenchymatous organs, small vascular spaces morphologically consistent with lymphatics were positive for D2-40. Such vessels were present in variable numbers in different organs. They were rare in the spleen (around central arteries and in the capsule), liver (in portal spaces and the capsule), kidney, and bone

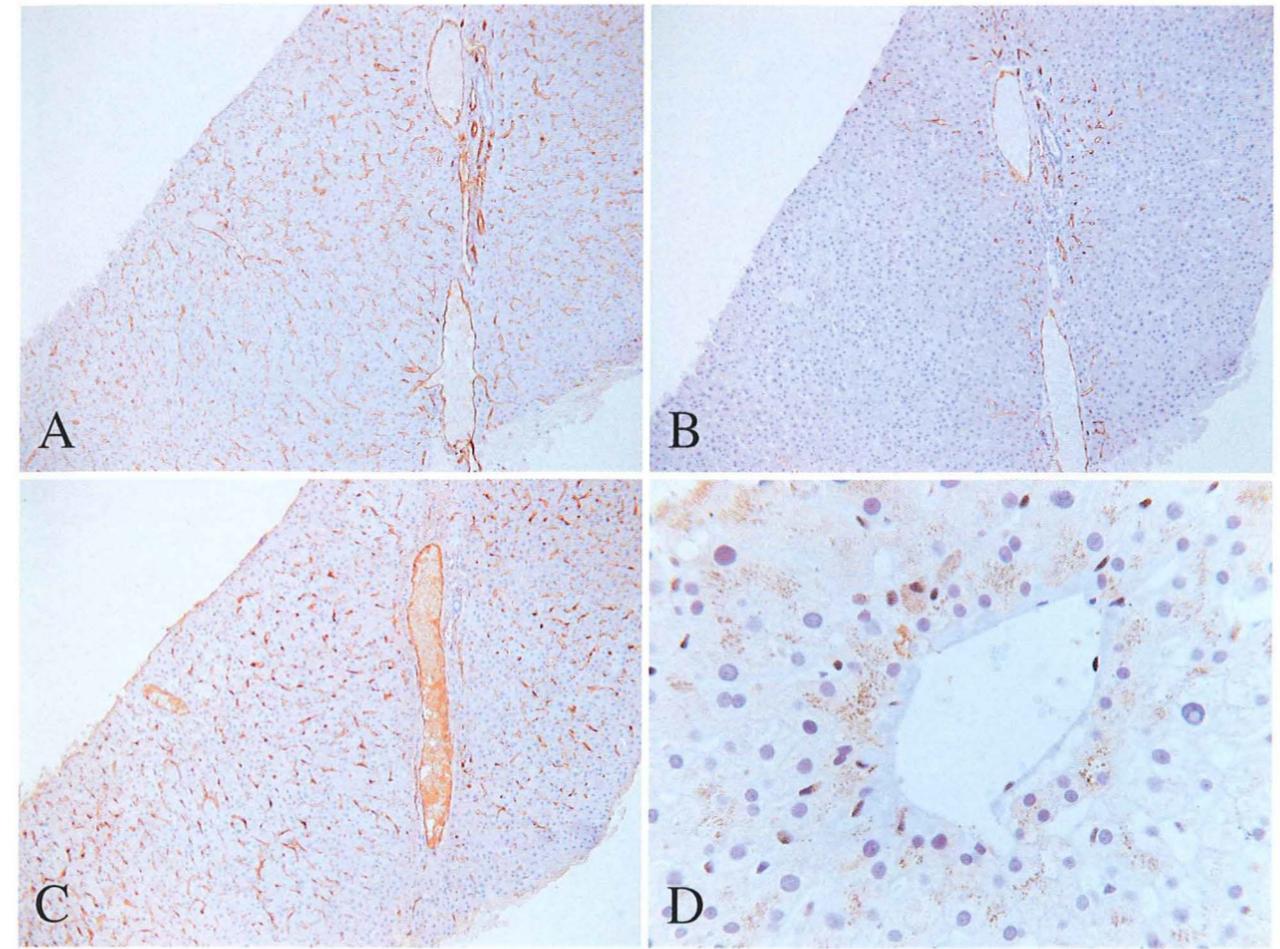


Figure 4 (A) CD31 expression in the liver showing diffuse and continuous positivity of the sinusoids all along the portal triad to the centrolobular vein. (B) CD34 expression in the liver showing focal positivity of the sinusoids, restricted to the periportal area. (C) vWF expression in the liver is similar to CD31. (D) Nuclei of EC from centrolobular vein and sinusoids are stained for Fli-1. A granular pattern of reactivity is also seen in the cytoplasm of hepatocytes.

marrow. In the lungs, they were usually seen around veins, arterioles, and bronchioles but were very rare or absent in the alveolar wall. In the heart, they were present in small numbers in between cardiomyocytes. Endocardial endothelium was negative for D2-40. Surprisingly, the number of D2-40-positive vessels present in lymph nodes was also low. The marginal and medullary sinuses were negative for D2-40. In the pericapsular region, D2-40-positive afferent and efferent lymph channels could rarely be identified. Few lymphatic vessels were present around arteries and veins in the adipous tissue around lymph nodes.

EC in arteries and veins were always negative for D2-40. CD31, CD34, vWF, and Fli-1 were occasionally and irregularly expressed in lymphatic endothelium, but the staining intensity was usually lower than in vascular endothelium and was very weak when compared with D2-40. Hence, D2-40 immunostaining gives a mirror image when compared with the other EC

markers (Figures 6A–6D). Mesothelial cells from the pleura or spleen capsule were occasionally positive for D2-40. This was also the case with osteocytes in the bone and bronchial wall chondrocytes, as well as basal cells from sebaceous glands and hair follicle cells, in a pattern similar to that of CD34. In bone marrow, hematopoietic stem cells including megakaryocytes were negative for D2-40.

Discussion

This study confirms that EC in different vessel types of various organs express EC antigens heterogeneously. Variations in expression patterns were most striking in the small capillaries of the continuous, fenestrated, and sinusoid type. We could confirm studies in humans (Gorog et al. 1998; Yamamoto et al. 1998; Müller et al. 2002c) and in animals (Rand et al. 1987) that show that the level of expression of vWF in the lung vasculature is

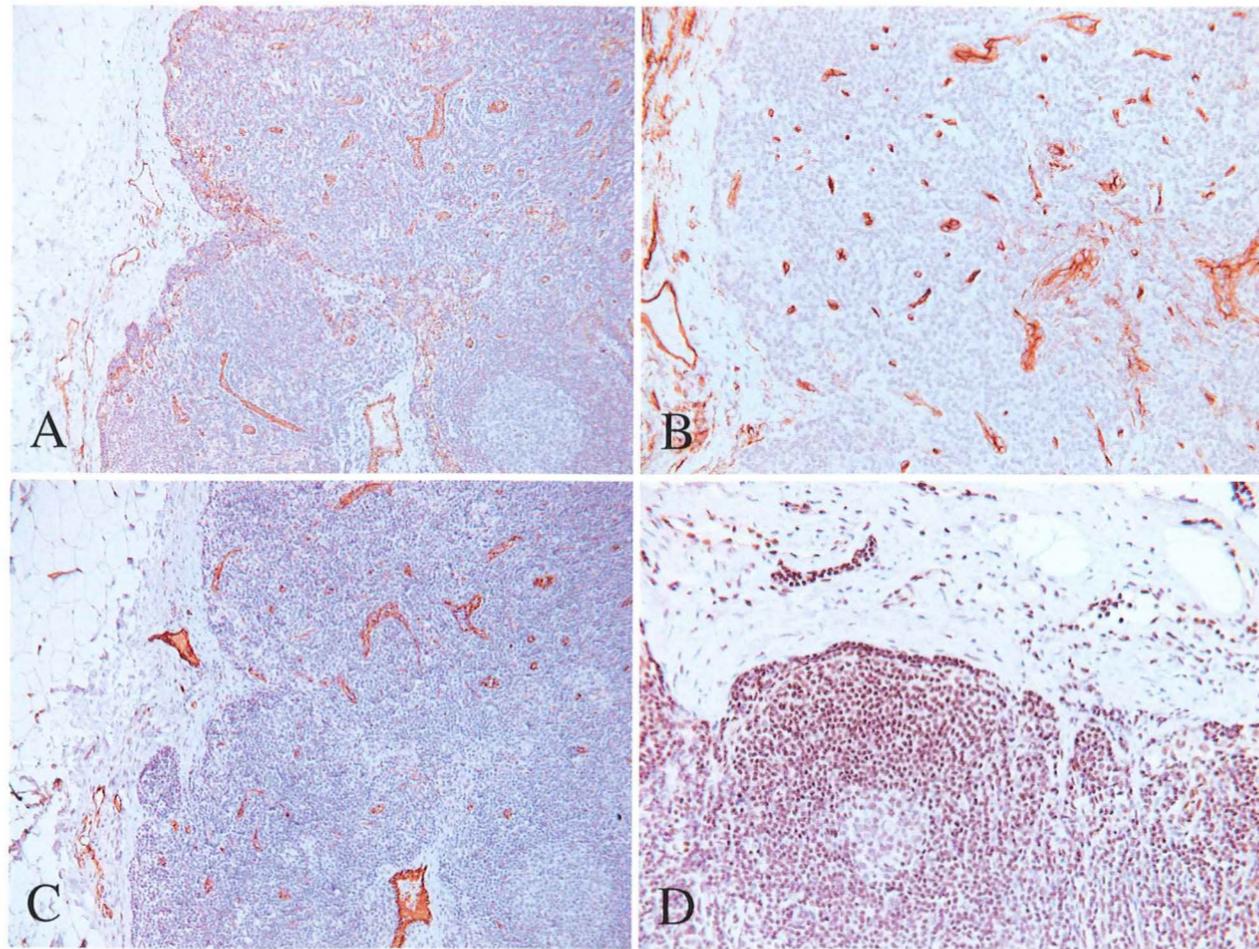


Figure 5 (A) CD31 expression in lymph nodes showing positive marginal (subcapsular) sinuses, high endothelial venules (HEV), and capillaries. In contrast, marginal sinuses are negative for CD34 (B) and vWF (C). (D) Most of the cells present in lymph nodes including EC from capillaries and HEV are stained for Fli-1.

higher in larger vessels (from being almost negligible in EC in alveolar capillaries but prominent in the major vessels). We also confirmed earlier findings in the kidney concerning immunoreactivity of fenestrated endothelium of the glomeruli for CD31 and CD34 but not for vWF and show, therefore, that antigen expression differences exist between EC in the same organ (Fleming and Jones 1989; Kinjo et al. 1989). We found that vWF expression was higher in renal glomeruli of patients with hypertension. This again confirms other studies that show upregulation of vWF expression on EC in hypertension and conditions such as acute respiratory failure (Carvalho et al. 1982), acute and chronic renal insufficiency, diabetic nephropathy (Lufkin et al. 1979), vasculitis, scleroderma, and Raynaud's phenomenon (Kahaleh et al. 1981). Increased levels of circulating vWF have also been described in patients with angina pectoris, coronary thrombotic events (Gorog et al. 1998), or pulmonary hypertension (Lopez and Maeda 1998).

In large vessels, discontinuous immunolabeling was found for CD31 and CD34, in spite of continuous labeling for vWF. This might be explained through loss of integrity of the EC lining, which was suggested by the presence of EC positive for CD31, CD34, and vWF inside the vascular lumen. Subendothelial matrix-bound vWF could explain the continuous staining seen with vWF (Rand et al. 1991). Smith et al. (1996) showed that vWF is differentially expressed in canine large vessels. We were not able to confirm these findings in normal human blood vessels.

CD34 also stained some components in the media of large vessels. Indeed, it has been shown in earlier studies that aortic smooth muscle cells and some fibroblasts also stain for CD34 (Miettinen et al. 1994).

We found D2-40 to be a sensitive and relatively specific marker for lymphatic endothelium in all parenchymatous organs. CD31, CD34, vWF, and Fli-1 did not discriminate between vascular and lymphatic EC and are therefore panvascular EC markers. How-

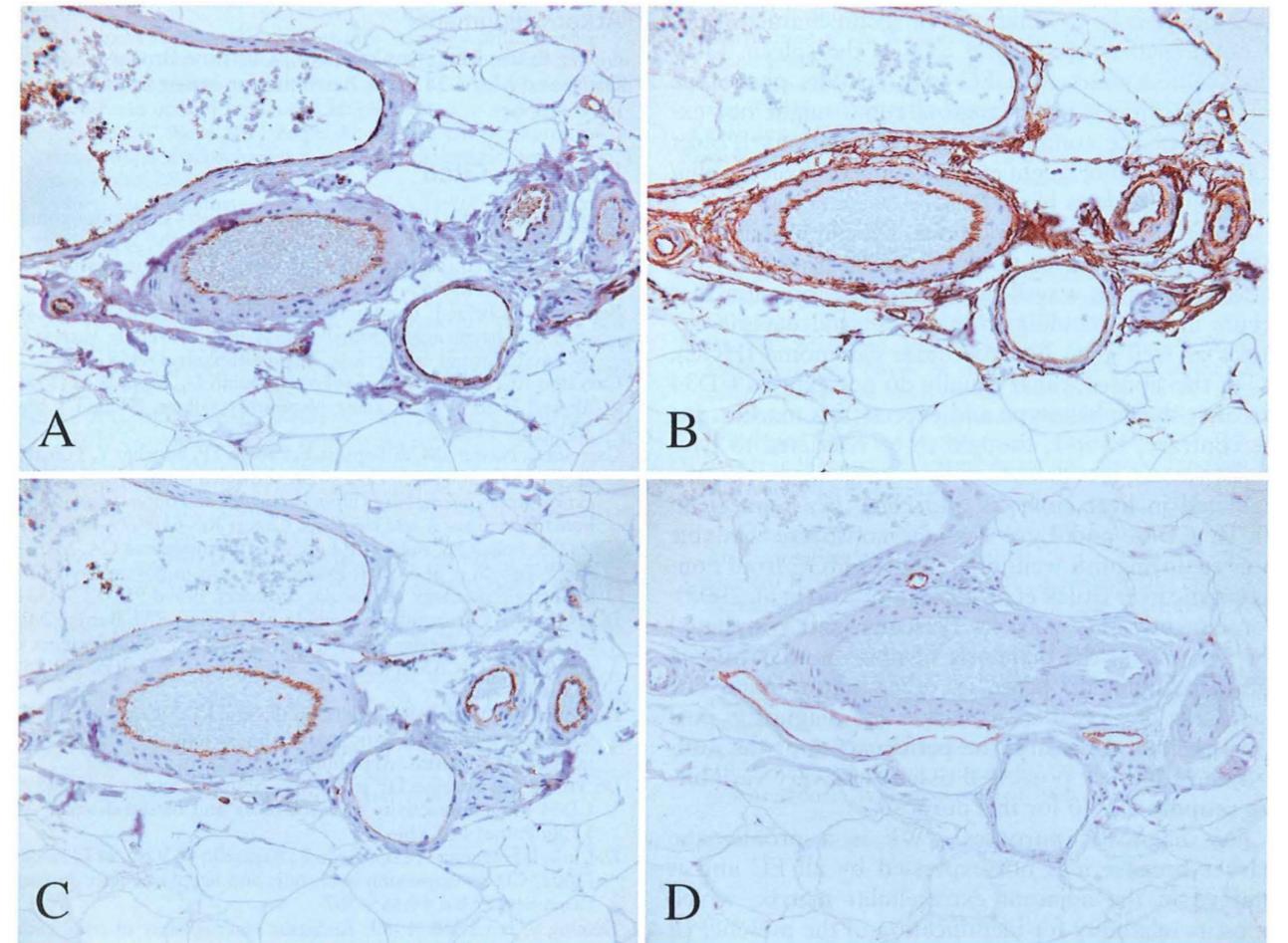


Figure 6 EC from arterioles and venules present in the skin are positive for CD31 (A), CD34 (B), and vWF (C), but negative for D2-40 (D). In contrast, lymphatic EC are strongly positive for D2-40 and weakly positive for the other markers. Adipocytes and interstitial cells around vessels are also positive for CD34.

ever, other cells (including mesothelial cells) also stained for D2-40. This is in agreement with a study showing that D2-40 is expressed in normal and reactive mesothelial cells and in most (93–96%) mesotheliomas (Ordonez 2005; Ordonez in press). D2-40 is also expressed in lymphangiomas, Kaposi's sarcomas, and a subgroup of angiosarcomas but not in endothelial cells of blood vessels. To our knowledge, the fact that osteocytes and chondrocytes also occasionally stain for D2-40 has not been reported previously. We can hypothesize that these D2-40-positive cells might express sialoglycoproteins similar to those of lymphatic EC and with which the antibody cross-reacts.

An important application of EC marker immunohistochemistry is the classification of human vascular tumors. CD31 is generally regarded as the single best marker of EC differentiation because it is expressed in ~90% of EC tumors but only very rarely in carcinomas, lymphomas, and mesotheliomas (DeYoung et al. 1998; Folpe et al. 2001). However, CD31 is also ex-

pressed in macrophages, which calls for careful interpretation of CD31 expression in poorly differentiated tumors (McKenney et al. 2001). CD34 is expressed in ~90% of vascular tumors. It is, however, also expressed in many other neoplasms, including dermatofibrosarcoma protuberans, malignant peripheral nerve sheath tumors, gastrointestinal stromal tumors, and epithelioid sarcomas. Notably, CD34 negative is the littoral cell angioma of the spleen, which correlates with the lack of expression of CD34 in the sinusoids of the spleen (Ben-Izhak et al. 2001). Korkusuz et al. (2002) identified the distinctive antigenic profile of sinus lining cells, in sharing both endothelial cell and macrophage lineage characteristics. In embryogenesis and vasculogenesis, vascular and hematopoietic tissues develop together from the early mesoderm (Cines et al. 1998). Hence, it is not surprising that the antigen expression pattern of hematopoietic cells and EC overlap; both cell types express CD31, CD34, vWF, and Fli-1. A common stem cell has been postulated that can differentiate into

hematopoietic or vascular cells or retain characteristics of both, such as the littoral cells of the spleen. Likewise, some tumors with EC stem cell-like phenotype or poorly differentiated angiosarcoma might not express all of the commonly used EC markers (Poblet et al. 1996) and/or might express some of the leukemia/lymphoma markers like CD34.

Under pathological conditions, EC can modify their antigen expression. In the liver, capillarization of hepatic sinusoids is a well-recognized phenomenon that occurs in long-standing liver disease and hepatic cirrhosis as well as in hepatocellular carcinoma (HCC). EC of the sinusoids that usually do not express CD34 can alter their phenotype and express this marker. On the contrary, Lyve-1, thought to be restricted to lymphatic vessels, is expressed in liver sinusoids but down-regulated in liver cirrhosis and HCC (Carreira et al. 2001). CD34 and Lyve-1 might constitute valuable tools to distinguish well-differentiated HCC from non-neoplastic liver (Ruck et al. 1995; Di Carlo et al. 2002). Consequently, EC markers (vascular and lymphatic) can also aid in the diagnosis of non-vascular tumors and of pathological processes other than tumors. The usefulness of Lyve-1 as a marker for diagnostic purposes is limited because the performance of the antibody on routinely processed tissues was very variable. We propose D2-40 for this purpose.

For diagnostic purposes, vWF is a problematic marker because it is not expressed by all EC and is retained in the adjacent extracellular matrix, which limits its reliability for identification of the presence of endothelial cells. In vascular tumors, it is expressed in only 50–75% of cases.

In our study, Fli-1 was expressed in all types of EC but was also expressed by other cell types, especially lymphocytes. The expression of Fli-1 did not change among different vessel types or organs. This marker can thus be a valuable tool in diagnosing both benign and malignant vascular tumors, especially when used in conjunction with membranous EC markers such as CD31 or CD34. The cytoplasmic reactivity seen in hepatocytes, renal tubular cells, and cardiomyocytes could be explained either by cross-reactivity with a cytoplasmic protein or by the presence of Fli-1 protein in the cytoplasm of these cells.

In conclusion, this study confirms that EC antigen expression varies among different vessel subtypes and different anatomic compartments of the same organ. The antigen expression pattern in a given organ or vascular bed is dynamic and can change due to a variety of pathological processes or simply in senescence (Müller et al. 2002b). Phenotypic differences of EC found in different vascular beds suggest highly differentiated site-specific functions of EC. This may be the result of specific characteristics of EC subtypes and a tissue-specific microenvironment.

Acknowledgments

We thank Magalie Cornavon, Christine Brueger, Solange Gros, and Marie-Martine Bertholet for expert immunocytochemistry support.

Literature Cited

- Alles JU, Bosslet K (1988) Immunohistochemistry of angiosarcomas. A study of 19 cases with special emphasis on the applicability of endothelial cell specific markers to routinely prepared tissues. *Am J Clin Pathol* 89:463–471
- Ben-Izhak O, Bejar J, Ben-Eliezer S, Vlodavsky E (2001) Splenic littoral cell haemangioendothelioma: a new low-grade variant of malignant littoral cell tumour. *Histopathology* 39:469–475
- Carvalho AC, Bellman SM, Saullo VJ, Quinn D, Zapol WM (1982) Altered factor VIII in acute respiratory failure. *N Engl J Med* 307:1113–1119
- Carreira C, Nasser SM, di Tomaso E, Padera TP, Boucher Y, Tomarev SI, Jain RK (2001) LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis. *Cancer Res* 61:8079–8084
- Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, Pober JS, et al. (1998) Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 91:3527–3561
- DeLisser HM, Christofidou-Solomidou M, Strieter RM, Burdick MD, Robinson CS, Wexler RS, Kerr JS, et al. (1997) Involvement of endothelial PECAM-1/CD31 in angiogenesis. *Am J Pathol* 151:671–677
- De Young BR, Wick MR, Fitzgibbon JF, Sirgi KE, Swanson PE (1993) CD31: an immuno-specific marker for endothelial differentiation in human neoplasms. *Appl Immunohistochem* 1:97–100
- De Young BR, Frierson HF Jr, Ly MN, Smith D, Swanson PE (1998) CD31 immunoreactivity in carcinomas and mesotheliomas. *Am J Clin Pathol* 110:374–377
- Di Carlo I, Frassetto F, Lombardo R, Azzarello G, Vasquez E, Puleo S (2002) CD 34 expression in chronic and neoplastic liver diseases. *Panminerva Med* 44:365–367
- Fleming S, Jones DB (1989) Antigenic heterogeneity of renal endothelium. *J Pathol* 158:319–323
- Folpe AL, Chand EM, Goldblum JR, Weiss SW (2001) Expression of Fli-1, a nuclear transcription factor, distinguishes vascular neoplasms from potential mimics. *Am J Surg Pathol* 25:1061–1066
- Fukunaga M (2005) Expression of D2-40 in lymphatic endothelium of normal tissues and in vascular tumours. *Histopathology* 46:396–402
- Gorog DA, Rakhit R, Parums D, Laffan M, Davies GJ (1998) Raised factor VIII is associated with coronary thrombotic events. *Heart* 80:415–417
- Hewett PW, Nishi K, Daft EL, Clifford Murray J (2001) Selective expression of erg isoforms in human endothelial cells. *Int J Biochem Cell Biol* 33:347–355
- Kahaleh MB, Osborn I, LeRoy EC (1981) Increased factor VIII/von Willebrand factor antigen and von Willebrand activity in scleroderma and Raynaud's phenomenon. *Ann Intern Med* 94:482–484
- Kawanami O, Jin E, Ghazizadeh M, Fujiwara M, Jiang L, Nagashima M, Shimizu H, et al. (2000) Heterogeneous distribution of thrombomodulin and von Willebrand factor in endothelial cells in the human pulmonary microvessels. *J Nippon Med Sch* 67:118–125
- Kinjo T, Takashi M, Miyake K, Nagura H (1989) Phenotypic heterogeneity of vascular endothelial cells in the human kidney. *Cell Tissue Res* 256:27–34
- Korkusuz P, Dagdeviren A, Asan E (2002) Immunophenotypic analysis of human spleen compartments. *Ann Anat* 184:431–441
- Kutzner H (1993) Expression of the human progenitor cell antigen CD34 (HPCA-1) distinguishes dermatofibrosarcoma protuberans from fibrous histiocytoma in formalin-fixed, paraffin-embedded tissue. *J Am Acad Dermatol* 28:613–617
- Lopez AA, Maeda NY (1998) Circulating von Willebrand factor antigen as a predictor of short-term prognosis in pulmonary hypertension. *Chest* 114:1276–1282

- Lufkin EG, Fass DN, O'Fallon WM, Bowie EJ (1979) Increased von Willebrand factor in diabetes mellitus. *Metabolism* 28:63–66
- Matsumura T, Wol K, Petzelbauer P (1997) Endothelial cell tube formation depends on cadherin 5 and CD31 interactions with filamentous actin. *J Immunol* 158:3408–3416
- McKenney JK, Weiss SW, Folpe AL (2001) CD31 expression in intratumoral macrophages: a potential diagnostic pitfall. *Am J Surg Pathol* 25:1167–1173
- Miettinen M, Lindenmayer AE, Chaubal A (1994) Endothelial cell markers CD31, CD34, and BNH9 antibody to H- and Y-antigens—evaluation of their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willebrand factor. *Mod Pathol* 7:82–90
- Müller AM, Hermanns MI, Skrzynski C, Nesslinger M, Müller KM, Kirppatrick J (2002a) Expression of the endothelial markers PECAM-1, vWf and CD34 in vivo and in vitro. *Exp Mol Pathol* 72:221–229
- Müller AM, Skrzynski C, Nesslinger M, Skipka G, Müller KM (2002b) Correlation of age with in vivo expression of endothelial markers. *Exp Gerontol* 37:713–719
- Müller AM, Skrzynski C, Skipka G, Müller KM (2002c) Expression of von Willebrand factor by human pulmonary endothelial cells in vivo. *Respiration (Herrlisheim)* 69:526–533
- Ordonez NG (2005) D2-40 and podoplanin are highly specific and sensitive immunohistochemical markers of epithelioid malignant mesothelioma. *Hum Pathol* 36:372–380
- Ordonez NG (In Press) The diagnostic utility of immunohistochemistry and electron microscopy in distinguishing between peritoneal mesotheliomas and serous carcinomas: a comparative study. *Mod Pathol*. Published online July 29, 2005 (DOI: 10.1038/modpathol.3800471)
- Poblet E, Gonzalez-Palacios F, Jimenez FJ (1996) Different immunoreactivity of endothelial markers in well and poorly differentiated areas of angiosarcomas. *Virchows Arch* 428:217–221

- Rand JH, Badimon L, Gordon RE, Uson RR, Fuster V (1987) Distribution of von Willebrand factor in porcine intima varies with blood vessel type and location. *Arteriosclerosis* 7:287–291
- Rand JH, Patel ND, Schwartz E, Zhou SL, Potter BJ (1991) 150-kD von Willebrand factor binding protein extracted from human vascular subendothelium is type VI collagen. *J Clin Invest* 88:253–259
- Rossi S, Orvieto E, Furlanetto A, Laurino L, Ninfo V, Dei Tos AP (2004) Utility of the immunohistochemical detection of FLI-1 expression in round cell and vascular neoplasm using a monoclonal antibody. *Mod Pathol* 17:547–552
- Ruck P, Xiao JC, Kaiserling E (1995) Immunoreactivity of sinusoids in hepatocellular carcinoma. An immunohistochemical study using lectin UEA-1 and antibodies against endothelial markers, including CD34. *Arch Pathol Lab Med* 119:173–178
- Smith JM, Meinkoth JH, Hochstatter T, Meyers KM (1996) Differential distribution of von Willebrand factor in canine vascular endothelium. *Am J Vet Res* 57:750–755
- Warhol MJ, Sweet JM (1984) The ultrastructural localization of von Willebrand factor in endothelial cells. *Am J Pathol* 117:310–315
- Weiss HJ (1991) von Willebrand factor and platelet function. *Ann NY Acad Sci* 614:125–137
- Westra WH, Gerald WL, Rosai J (1994) Solitary fibrous tumor. Consistent CD34 immunoreactivity and occurrence in the orbit. *Am J Pathol* 142:992–998
- Yamamoto K, de Waard V, Fearn C, Loskutoff DJ (1998) Tissue distribution of von Willebrand factor gene expression in vivo. *Blood* 92:2791–2801
- Yamamoto M, Shimokata K, Nagura H (1988) An immunohistochemical study on phenotypic heterogeneity of human pulmonary vascular endothelial cells. *Virchows Arch A Pathol Anat Histochem* 412:479–486
- Zhou Z, Christofidou-Solomidou M, Garlanda C, DeLisser HM (1999) Antibody against murine PECAM-1 inhibits tumor angiogenesis in mice. *Angiogenesis* 3:181–188