Structural and immunophenotypic characterization of high endothelial venules in rat and human tissue

Murat Akkus, *PhD,* ***Sinan Yuruker,*** *MD,* ***Attila Dagdeviren,*** *MD,* ***Esin Asan,*** *PhD.*

ABSTRACT

Objective: To present additional data on high endothelial venule (HEV) structure and immunophenotype.

Methods: We used the zinc iodide-osmium tetroxide technique (ZIO), which is a metallophilic fixation and staining technique to examine HEVs at light and electron microscopic levels as this technique was previously reported to be reactive with cells in HEVs. Tonsils and lymph nodes were obtained from the Surgery and Otorhinolaryngology Departments, Hacettepe University Hospital, Ankara, Turkey during 2002 and 2003. An indirect immunohistochemical technique was used to examine frozen human tissue samples.

Results: Organelle rich high endothelial cells, sheet-like processes of pericytes surrounding HEVs, structural relation of pericyte processes with fibroblastic reticular cells, an unusual multivesicular body-like organelle within high endothelial cells were presented. Expression of a large panel of defined and yet non-defined antigens on HEVs are also presented using an indirect immunoperoxidase technique.

Conclusion: Presence of some of these antigens on HEVs was previously reported while no previous report is available for others. Significance of the expression of these antigens in HEVs, structural hints for trans endothelial migration of lymphocytes and their travel along the reticular cell meshwork is briefly discussed.

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Recruitment of immune system cells, predominantly lymphocytes, to tissues or organs is a crucial step of immune response to be developed. Thus, high endothelial venules (HEVs), providing a special pathway for lymphocyte migration from peripheral blood into secondary lymphoid tissues or various body compartments (usually under pathological conditions), is of special interest. Though HEVs are first described more than a hundred years ago,[1] and named by Schulze[2] in 1925 , they are recognized to be a selective site for lymphocyte migration in 1959 by Gowans.[3] Since then, numerous studies carried out to understand how this process takes place, what controls the selective migration through HEVs structurally and functionally.[4-8] Accumulating findings revealed that an adhesive interaction between immune system cells (lymphocytes, NK cells, dendritic cells and so forth) and high endothelial cells (HECs) were found to be the key process in this selective migration as reviewed by Stamenkovic.[9] A number of cell adhesion molecules and their ligands (mostly termed as homing receptors or addressins) possibly involved in this process are then detected. Among major adhesion molecules participating in the lymphocyte traffic

From the Department of Histology and Embryology (Akkus), Dicle University Faculty of Medicine, Diyarbakır and the Department of Histology and Embryology (Yuruker, Dagdeviren, Asan), Faculty of Medicine, Hacettepe University, Ankara, *Turkey*.

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Address correspondence and reprint request to: Dr. Sinan Yürüker, Assistant Professor, Department of Histology and Embryology, Faculty of Medicine, Hacettepe University Sıhhiye, 06100 Ankara, *Turkey*. Tel. +90 (312) 3052145. Fax. +90 (312) 4780168. E-mail: sinany@hacettepe.edu.tr

through HEVs; CD49d, L-selectin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) are well documented examples.[10] Though possibly a number of adhesion molecules are involved in this multi-step process there are many studies directed to L-selectin (CD62-L) expressed by lymphocytes and its carbohydrate ligands on high endothelial cells because this receptor or ligand pair is involved in the initial steps (rolling and tethering of leukocytes on endothelial cells) of lymphocyte homing. Selectin ligands are a set of mucin-like glycoproteins and glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1) is a candidate to be such a molecule. 6-sulfo sialyl Lewis(x) on L-selectin binds to counter-receptors CD34, GlyCAM-1, and MAdCAM-1. Recently, enzymes involved in the sulfation and fucosylation of carbohydrate moieties are determined and some of them are cloned as these modifications on carbohydrate ligands results in a selectivity in lymphocyte homing.[11,12] As mentioned above homing of leukocytes is a multi-step process not limited to L-selectin and its ligands thus investigators keep seeking for other HEV specific molecules which are possibly involved in the yet not completely understood but rather selective process. For example, lymphocyte binding to HEV in non-inflamed peripheral lymph nodes (PLN) relies heavily on 2 endothelial adhesion molecules called vascular adhesion protein-1 (VAP-1) defined by mAb 1B2 and the peripheral lymph node addressins (PNAd) defined by mAb MECA-79.[13] A recently identified HEV related molecule possibly involved in some steps of transmigration through HEVs is angiomodulin (mac25/AGM). Usui et al[14] reported that mac25/AGM is an activated endothelial cell marker expressed on abluminal surface of high endothelial cells possibly involved in capturing humoral factors produced in the vicinity of HEVs to modulate further activation of endothelial cells allowing to transmigration.[14] Another recently identified HEV specific molecule is the vascular endothelial-junctional adhesion molecule (VE-JAM)/JAM-2, which is shown to be interacting T, NK, and dendritic cells through JAM-3 for trafficking into lymph nodes and inflammatory sites.[15] Leucin rich HEV glycoprotein (LRHG) is another recently determined molecule specific to HEVs possibly functioning in the binding of HECs to extracellular matrix components such as fibronectin, collagen IV, and laminin. In addition, LRHG binds TGF-ß reflecting a multifunctional molecule property.[16] Yet, another family of molecules seemingly participating in leukocyte homing is chemokines. A member of this family; secondary lymphoid tissue chemokine (SLC) has been shown to participate in binding of lymphocytes to HEVs via ß2-integrin/ICAM-1 receptor/ligand pair.[17] In this study, we present additional information on both structure and antigenic profile of HEVs for future directions using 2 distinct techniques.

Methods.

Light and electron microscopic examination. Adult Wistar rats weighing 150-200 g (n:6) were used to obtain tissue samples. Animals were killed by decapitation under ether anesthesia and submandibular lymph nodes were quickly removed.

Processing with the Niebauer Zinc iodide-osmium (ZIO) technique. Freshly obtained tissues were immersed in the ZIO solution used by Niebauer et al. (1969) and kept in the dark for 24 hours in this solution at room temperature. The Niebauer solution is prepared by mixing 2g metallic iodine and 6g metallic zinc powder with 8ml distilled water. This is added to 80ml bi-distilled water slowly, as it is an exothermic reaction. The solution is filtered after 5 minutes to remove excess zinc and is added to an unbuffered 2% solution of osmium tetroxide at a ratio of 4 parts to one. It is prepared fresh and kept in the dark before use. The final pH of the unbuffered ZIO solutions was measured and found 5.5 $\pm$ 0.3. Fixed/stained tissues were processed for routine electron microscope analysis. Semi-thin and thin sections are obtained from plastic embedded blocks, examined and photographed by Olympus BH2 light microscope at light microscopic level; Zeiss-EM9-S2 at electron microscopic level.

Immunohistochemical examination. Tissues. Tonsillectomy material (operated due to chronic sore throat; n:5) was obtained from the Otorhinolaryngology Department; partial lymph node biopsy specimens (operated for various reasons; n:4) were obtained from the General Surgery Department of Hacettepe University Hospital, Ankara, Turkey during August 2002 to February 2003. All tissue specimens were immediately frozen in liquid nitrogen and stored at -30 °C. Cryostat sections (6-8 µm thick) were taken and placed on gelatin-coated slides and kept in humidity-free containers at room temperature, and the staining was carried out within a week.

Antibodies and staining procedure. Primary monoclonal anti-bodies (mAbs) used in this study are listed in **Table 1**. They were supplied from the Vth Leukocyte Typing Workshop, Boston; VIth Leukocyte Typing Workshop, Kobe and VIIth Leukocyte Typing Workshop, Harrogate. Indirect immunoperoxidase procedure used in the study has been described elsewhere.[19] Sections were fixed in acetone for 10 minutes and air-dried for at least 30 minutes. Sections were then incubated for 60 minutes with mAbs. Dilutions used of each primary antibody were shown in **Table 1**. After washing in 0.01 M phosphate buffered saline (PBS) pH 7.4, the slides were covered with a 1:200 dilution of rabbit

anti-mouse immunoglobulin G peroxidase (Sigma, Cat No: B-9904, St. Louis) in PBS containing 0.2% bovine serum albumin (Sigma Cat No: A-7034, St. Louis) and 1% normal human serum. After washing in PBS, the slides were stained for peroxidase activity with 3.3'-diaminobenzidine-tetrahydrochloride (Sigma Cat No: D-5637, St. Louis) (0.5 mg/ml Tris-HCl buffer, pH 7.6, containing 0.01% H_2O_2). Counterstaining with hematoxylin was carried out. The control staining was performed by both omitting the first (primary antibody) step and using an irrelevant mouse primary antibody. Stained sections were examined and photographed using an Olympus BH2 light microscope.

Results. ***Light microscopic findings.*** In the examination, ZIO stained and toluidine blue counterstained semi-thin sections of rat lymph nodes, HEVs are easily distinguished with mostly ZIO reactive endothelial cells (**Figure 1a**). Pericytes, fibroblastic reticular cells and macrophages were also determined to be ZIO (+) cells in the tissue (**Figure 1b**). The lumina of HEVs are usually too narrow being distinguished by the presence of a few erythrocytes or white blood cells. However, some HEVs with a larger lumen were also present as distinguished by the migrating lymphocytes along their wall (**Figure 1c)**. High endothelial venules were observed mostly in the deep cortical region.

Table 1 - Expression of certain antigens in the vasculature of human lymphoid organs.

CD code of the antigen	Alternative name	Clone name of mAb	HEV	A-En	V-En	C-En	S-En
CD 29	VLA-_1	TS2/16	+++	+++	+++	+++	++
CD 31	PECAM-1	JC70A	++++	++++	++++	+++	+/--
CD 34	HA-receptor	QBEnd10	+++	++	++	++	++
CD 39	Ecto-apyrase	B721	++	+	+	+	-
CD 49a	VLA-_1	SR84	++	+++	+++	+++	+++
CD 49b	VLA-_2	6F1	+/--	+/--	+/--	-	-
CD 49c	VLA-_3	J143	+++	++	++	-	-
CD 49d	VLA-_4	8F2	+/--*	-	-	-	-
CD 49e	VLA-_5	SAM-1	++	+++	++	++	-
CD 49f	VLA-_6	BQ16	++†	+++	+++	+++	+++
CD 54	ICAM-1	CBR-IC1/3	++++	+	++	+	+/-
CD 62E	E-selectin	4D10	++*	-	++*	-	-
CD 62P	P-selectin	G1	+++‡	-	++†	-	-
CD 71	Transferrin-R	AC108	+	+	+	+	
CD 98	4F2	2E12	++	++	++	+	-
CD100	SEMA4D	A8	+	+	+	+	-
CD102	ICAM-2	CBR-IC2/1	++++	++	+	++	+++
CD105	Endoglin	44G4	++++	++	++	++	+
CD106	VCAM-1	E1/6	+/-	+/-	+/-	-	-
CD109	8A3	8A3	++	++	++	++	-
CD143	ACE	9B9	+	+	+	+	
CD146	MUC-18	F435H-7	+++	++	++	++	++
CD147	Neurothelin	UM-8D6	++	-	-	-	
CD151	PETA-3	B-F45	+++	++	++	++	
CD202b	Tie-2/TEK	B-H38	+	++	++	++	
CD231	TALLA-1	SN1	+++	++	++	++	
-	-	VR148	+++	++	++	+	+
-	-	MH46	++	++	++	++	++
-	-	14A2.H1	++	++	++	-	-
-	-	B-D46	+	+	+	+	
-	-	B-C44	++	+++	+++	+++	
-	-	HIE4D1	++	+	+	+	
-	-	SCF87	+++	-	++	++	
-	-	LIA1/17	+	++	++	++	
-	-	VJ1/6	++	++	++	++	
-	-	8B4/20	++	+	+	+	
-	-	A3	+	-	-	-	

HEV - high endothelial venule, A-En - artery/arteriole endothelium, V-En - vein/ venule endothelium, C-En - capillary endothelium
S-En - sinusoidal endothelium, ++++ = too strong, +++ = strong, ++ = intermediate, + = positive,
+/- = weak, +/-- = too weak reaction or expression, *subgroup, †basal reaction, ‡cytoplasmic reaction

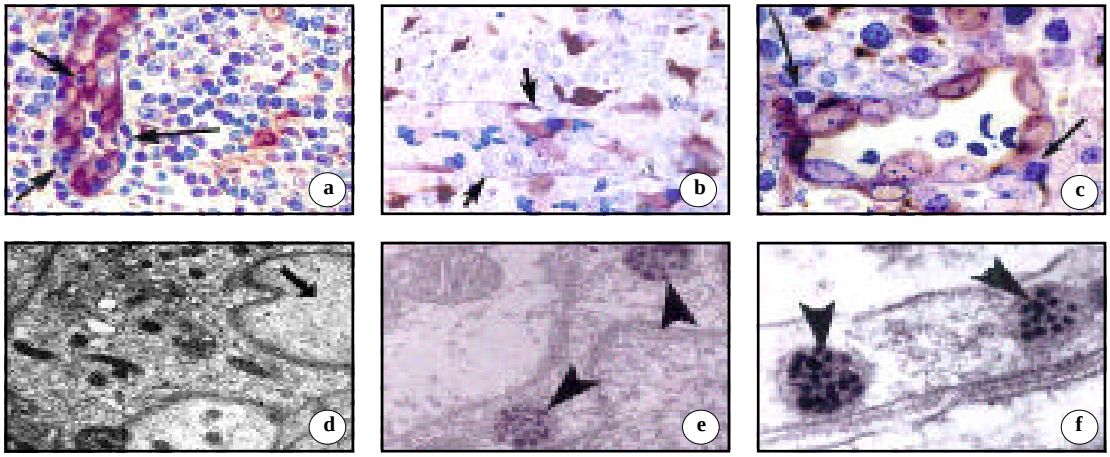

Figure 1 - Photograph of **a)** A high endothelial venule with mostly zinc iodide-osmium (ZIO) (+) endothelial cells in the paracortical area of a rat lymph node is seen. The lumen of the HEV is rather narrow distinguished by the presence of few erythrocytes within it. Several migrating lymphocytes are indicated with arrows. Zinc iodide-osmium tetroxide fixed and stained, toluidine blue counter stained, original magnification x 20. **b)** Another high endothelial venule with similar structural features is seen. High endothelial cells are ZIO (-). Migrating lymphocytes are indicated with arrows. Pericytes and dendritic cells/macrophages are ZIO (+). Zinc iodide-osmium tetroxide fixed and stained, toluidine blue counter stained, and original magnification x 40. **c)** Another HEV with a large lumen is seen under higher magnification. This venule is distinguished as a HEV with a few migrating leukocytes indicated by arrows. Zinc iodide-osmium tetroxide fixed and stained, toluidine blue counter stained, and original magnification x 100. **d)** Electron micrograph of a high endothelial cells. Nucleus (N) and large cytoplasmic compartment rich in organelles is seen consisting of several mitochondria, granular and smooth endoplasmic reticulum cisternae or vesicles, free ribosomes, and part of Golgi apparatus. Uranyl acetate-lead citrate stained x 5500. **e)** High power micrograph of an high endothelial cell consisting of 2 vesicles with a ZIO (+) multilocular content resembling a special form of a multivesicular body. Uranyl acetate-lead citrate stained x 25000. **f)** Two such organelles are seen at a higher magnification. This membrane bound organelle has mostly rounded and some irregularly shaped osmiophilic material. Uranyl acetate-lead citrate stained x 35000.

However, they were also present in more superficial compartments of lymph nodes between follicles. Number of HEV sections was relatively higher in the lymph nodes, which are rich in follicles at their cortical compartment. Parallel to this finding, number of migrating lymphocyte figures along the thick HEV walls was also higher in such lymph nodes. Lymphocytes were always ZIO (-) thus, to distinguish the migrating lymphocytes through HEVs was easier.

Electron microscopical findings. Electron microscopic examination of HEVs revealed that endothelial cells of these unique vessels are extremely rich in organelles (**Figure 1d**). Nucleus was euchromatin rich with a prominent nucleolus. Extensive figures of endoplasmic reticulum cisternae (mostly granular and some smooth), several electrons dense elongated mitochondria, free ribosomes, cisternae of Golgi complex were observed in the cytoplasm of HECs. At higher magnification, an unexpected membrane bound organelle was also determined with a multilocular ZIO (+) content (**Figure 1e & 1f**). As in light microscopical examination most of the HECs were ZIO (+) at varying degrees. Interestingly sections of small blood vessels approximately at the size of capillaries surrounded by large pericytes were observed. In contrast to its small caliber these, vasculature was lined by several endothelial cells with a more or less cuboidal outline when compared to flattened lining of classical capillaries (**Figure 2a**). At higher magnification of peripheral compartment of HEVs, layers of pericytes surrounding these vessels were clearly outlined (**Figure 2b**). Most of the pericytes and their processes were ZIO (+) and they are surrounded by a prominent external (basal) lamina. Migrating lymphocytes at the abluminal compartment of HEV wall were recognized forming rows between HECs and first layer of pericytes or between pericyte processes of outer layers (**Figure 2c**). Zinc iodide-osmium (+) processes at these peripheral locations helped to distinguish the structural relation of these processes with the lateral compartment of HEVs consisting of newly migrated lymphocytes, which profiles were reflecting the migratory pattern (**Figure 2d**). In some sections, relation of pericyte processes with the reticular meshwork was also clearly observed (**Figure 2e**).

Immunohistochemical findings. Results of the immunohistochemical examination are summarized in **Table 1** as a list of HEV reactive mAbs. Most of the antigens expressed on endothelial cells are covered by CD codes. However, certain antigens expressed on endothelial cells, which are not yet

covered by CD codes are also included in the panel examined. A group of antigens are expressed strongly on the endothelium of all types of blood vessels including sinusoidal endothelium: VLA integrin-α1 (CD49a), -α6 (CD49f) and common ß1 chains (CD29); hyaluronan receptor (CD34); ICAM-2 (CD102), Endoglin (CD105); MUC18/S-Endo (CD146), mAbs MH46 and VR148 (**Figures 3a-3h**). Reactivity of CD49f was significantly basally located on endothelial cells (**Figure 3b**). Similarly, PECAM-1 (CD31) and ICAM-1 (CD54) are also expressed by all types of endothelial cells but weaker on sinusoidal endothelium (**Figure 3c**). Several antigens were also expressed by all types of vascular endothelial cells except sinusoidal endothelium including; CD39, VLA-α3 chain (CD49e); transferrin receptor (CD71); 4F2 antigen (CD98); CD100; CD109, CD143, PETA-3 antigen (CD151); Tie2/TEK (CD202b), TALLA-1 (CD231), mAbs B-D46; B-C44; HIE4D1; LIA1/17; VJ1/6 and 8B4/20. Several antigens were expressed by only HEV/artery/vein endothelia being absent on capillaries and sinusoids such as VLA-α2 chain (CD49b); -α3 chain (CD49c); VCAM-1 (CD106) and mAb 14A2.H1. Selectins CD62E and CD62P are expressed by only HEV and vein endothelia. Expression of CD62E was variable being present in subgroup of these vasculature and expression of CD62P was cytoplasmic or granular differing from the other expression patterns (**Figure 3d**). Few antigens examined were only reactive with HEVs including CD147, VLA-α4 chain (CD49d) and mAb A3. Among these only CD147 is expressed prominently. The reaction for A3 mAb was moderate and was too weak in some HEVs for CD49d. Most of the antigens are expressed by endothelial cells are also expressed by subendothelium or vascular smooth muscle as well.

Discussion. Presence of tall endothelial cells and migrating lymphocytes through their wall helps the recognition of HEVs under light or electron microscope using routine techniques. However, to study their distribution in lymphoid organs or in other tissue compartments under pathological conditions specific markers will certainly be beneficial. Such histochemical or immunohistochemical techniques also provide a useful tool to determine their relation with surrounding tissues. The presence of HEV-like vessels in unexpected locations like interstitium of kidneys in glomerulonephritis is previously reported. High endothelial venules are characterized by using markers against HEV related molecules such as P-selectin and L-selectin ligands; MECA-79 epitope and variant sulfated forms of sialyl Lewis X (variant sLe(X), clones 2H5, 2F3,

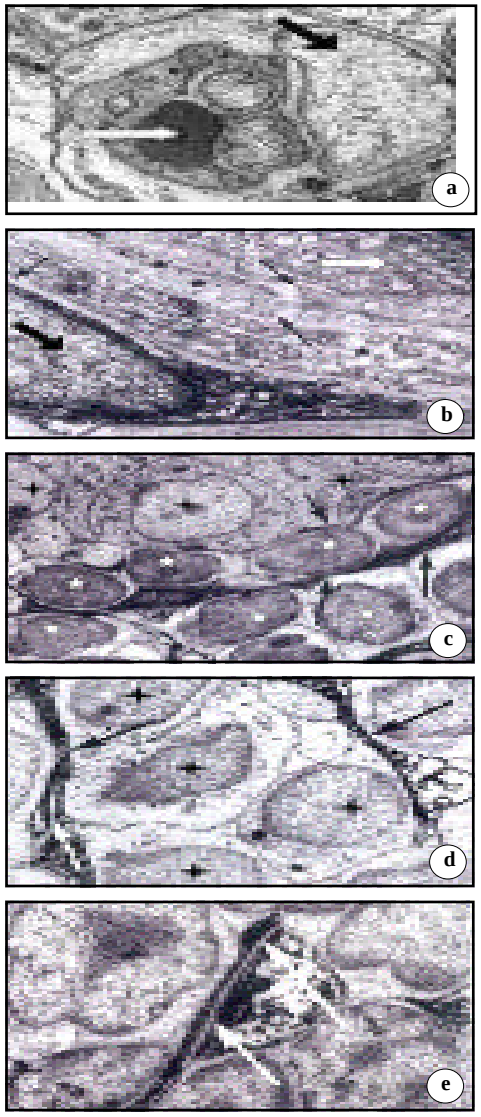

Figure 2 - Photograph of **a)** A pericyte surrounding a small blood vessel lined by 3 endothelial cells in rat lymph node is seen. An erythrocyte (white arrow) in the lumen helps to distinguish the size of this developing blood vessel. Uranyl acetate-lead citrate stained, x 5500. **b)** Peripheral compartment of the HEV wall. High endothelial cell (white arrow), layers of pericyte processes (*) and a zinc iodide-osmium (ZIO) pericyte cell body with the nucleus (thick black arrow) and perinuclear cytoplasm are seen. Sheet-like processes of pericytes are surrounded by external lamina (thin arrows). Uranyl acetate-lead citrate stained x 11000. **c)** Migrating leukocytes at the peripheral compartment of a HEV is seen. High endothelial cells (+) are extremely rich in organelles, migrating lymphocytes are observed along mostly ZIO (stars) pericytic processes (arrows). Two rows of migrating lymphocytes are observed. Uranyl acetate-lead citrate stained x 7500. **d)** Higher magnification of the peripheral pericytic compartment with processes (arrows) and lymphocytes (+). Uranyl acetate-lead citrate stained, x 6500. **e)** A ZIO (+) pericyte process is seen under higher magnification. Arrows indicate the extracellular matrix elements (reticular fibers) surrounded by the process. Uranyl acetate-lead citrate stained x 8000.

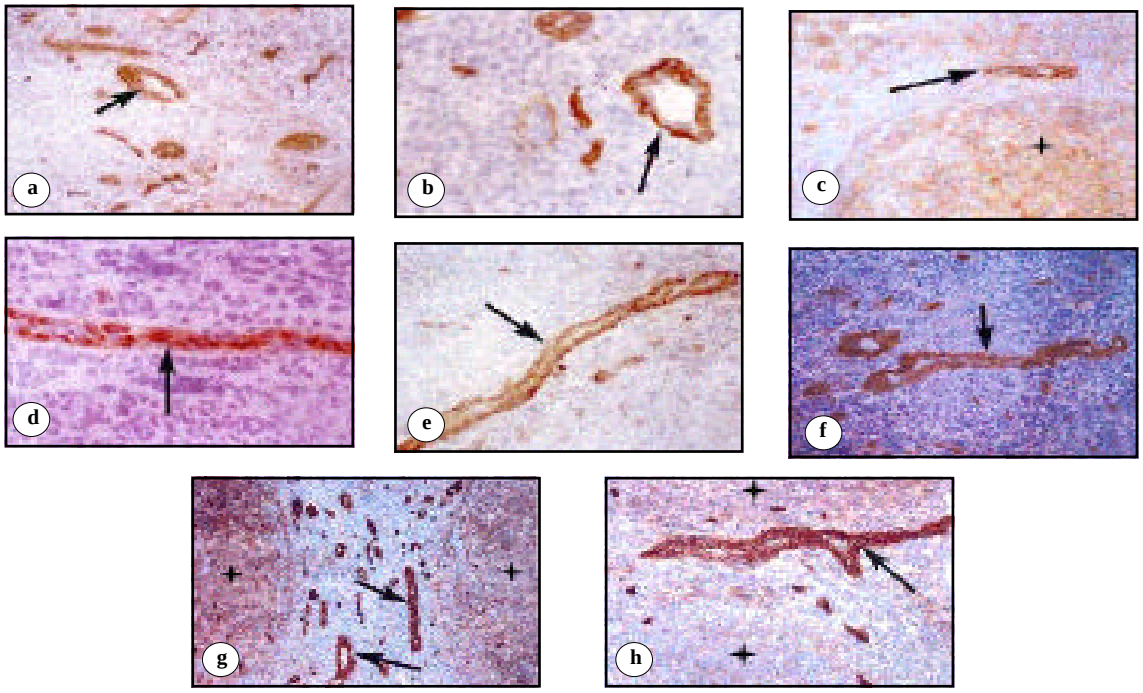

Figure 3 - Photograph of **a)** A palatine tonsil section immunostained with anti-CD34 monoclonal antibody. All types of blood vessels are CD34 (+) including high endothelial venules (HEVs) (arrow). Indirect immunoperoxidase stained, hematoxylin counterstained, original magnification x 20. **b)** Interfollicular area of palatine tonsil immunostained for VLA-6 achain (CD49f). Several capillaries and 2 high endothelial venules are seen. Reactivity was localized mostly to the basal compartment of HEV wall (arrow). Indirect immunoperoxidase stained, hematoxylin counterstained, original magnification x 40. **c)** CD54 (ICAM-1) reactivity in palatine tonsil. HEV and follicular dendritic cells of a lymphatic follicle are CD54 (+). Indirect immunoperoxidase stained, hematoxylin counterstained, original magnification x 20. **d)** CD62P (P-selectin) reactivity in a HEV located in the interfollicular area of palatine tonsil. Granular cytoplasmic character of the CD62-P reactivity is distinguished. Indirect immunoperoxidase stained, hematoxylin counterstained, original magnification x 40. **e)** CD105 (endoglin) antigen was strongly expressed by HEV cells. A relatively weaker expression was present in other vascular elements is seen. Indirect immunoperoxidase stained, hematoxylin counterstained, original magnification x 20. **f)** CD109 (+) 2 HEVs are seen. Indirect immunoperoxidase stained, hematoxylin counterstained, original magnification x 20. **g)** Low power micrograph showing CD146 (MUC-18) reactivity in tonsil. All blood vessels including HEVs are strongly reactive for the antigen. Follicular dendritic cells of the adjacent follicles are also moderately reactive (f). Indirect immunoperoxidase stained, hematoxylin counterstained, original magnification x 10. **h)** Higher magnification of a follicle and adjacent interfollicular area immunostained for CD146 antigen. Vasculature including a HEV (arrow) and follicular dendritic cells in a follicle (+) are seen. Indirect immunoperoxidase stained, hematoxylin counterstained, original magnification x 20.

GS-13 and GS-36), P-selectin and hevin mRNA signals by using in situ hybridization analyses in this study.[20] Chondroitin sulfate proteoglycan at the basal lamina beneath high endothelial cells in human palatine tonsils is shown using colloidal iron method in another study.[21] Reactivity of ZIO technique in HEVs in rat was previously reported by Dagdeviren et al.[22] Thus, we used this technique to examine light and electron microscopic examination of HEVs in rat for further structural evidences. Using this technique distribution and structural features of HEVs were determined clearly though the reason for the specificity of the technique is not yet assigned. In addition to most HECs, fibroblastic reticular cells, pericytes and macrophages were also reactive for ZIO. Migrating lymphocytes were detected more easily as they were all ZIO (-) traveling along mostly ZIO HECs and pericyte processes. Following migration through HEV wall, lymphocytes appeared to be occurring in rows guided by pericyte processes at the periphery of HEVs before entering the spaces of fibroblastic reticular cell meshwork. Some of the extensive studies on lymph node microanatomy were directed to structural relation of fibroblastic reticular cell (FRC) network and HEVs regulating lymphocyte traffic from blood to lymph nodes.[23-25] Concepts of corridors, chords, channels and conduit were introduced and functional significance of these compartments were reviewed by Gretz et al.[26,27] Our electron microscopic findings support the definitions of these studies. Above mentioned organization of migrating lymphocytes in rows along pericyte processes indicate the relation of migratory path of lymphocytes through HEVs into pericyte or FRC network. Additionally, we observed tiny pericytic and FRC processes enclosing extracellular matrix elements (reticular fibers),

reflecting the existence of conduits as minute spaces structurally related to the peripheral compartments of HEVs. In a previous report, it is stated that there is evidence that high endothelial venule cells phagocytose apoptotic leukocytes following corticosterone treatment thus participate in the removal of apoptotic cells in addition to macrophages.[28] We did not detect any sign of lymphocyte phagocytosis by HECs in our extensive examinations but ingested lymphoid figures were seen in follicular macrophages. However, we observed a multivesicular body-like organelle with a ZIO (+) content in HECs. Though multivesicular bodies are generally accepted as a component of phagocytic machinery in general, we suggest that this yet undefined multivesicular body-like organelle is related to vesicular traffic (receptor endocytosis) rather than reflecting a phagocytic activity of HECs directed to lymphocytes. Route of lymphocyte migration through the high endothelial venules is still not clear as findings favoring interendothelial or transendothelial migration are both reported by different investigators. Indrasingh et al[29] reported that they observed signs of both migration pathways. We mostly observed migrating lymphocytes entirely surrounded by HEC cytoplasm rather than interendothelial location. Thus, our observations support the transendothelial migratory path for lymphocytes through HECs but also cannot certainly discard interendothelial route.

The goal of our immunohistochemical examination on HEVs was determining the HEV reactive mAbs that could be used as a marker, rather than obtaining functional evidence on the expressed molecules, screening a wide range of mAbs. Strong expression of certain antigens on HEVs was reported previously such as endoglin (CD105), VLA integrin chains, ICAM-1 (CD54), ICAM-2 (CD102), MUC18/S-endo (CD146) and so forth.[30-32] Most of these antigens were also expressed strongly on the endothelium of all types of blood vessels including sinusoids and some by vascular smooth muscle as well. Another cell type commonly sharing antigens with HECs is follicular dendritic cells (FDCs). As they exist on HECs these antigens carry out specific functions in HEVs but evidence for most of them is still lacking remaining to be elucidated with the exception of well studied HEV-related molecules such as selectins, and ICAMs. Among the antigens examined those reactive mainly with HEVs lacking reactivity on other endothelial cells or cell types were of special interest as they represent selective markers for HEVs possibly carrying out HEV specific functions. Among these CD147, and the yet undefined antigen detected by mAbs SCF87 (being absent on arterial endothelia but present on vein and capillary endothelium) and A3 (with a weaker reactivity) were remarkable ones. However, we also believe that certain antigens such as endoglin, ICAMs, CD31, CD34, some integrins CD105, CD146 and so forth. provide useful tools to examine HEVs as a marker being strongly reactive with HECs though they are broadly expressed by certain stromal cell types. Although there are numerous attempts to determine such specific markers it should also be considered that it is rather hard to define a true HEV specific molecule as ongoing studies might reveal the expression of these antigens on cell types other than HECs. Hevin is a good example for such a case. This molecule is determined as a HEV protein being absent in flattened endothelial cells.[33] However, SC1/Hevin is found to be associated with collagen type-I fibers; thus, this extracellular calcium-modulated protein is suggested to be involved in collagen binding and broadly expressed by a variety of cells in a recent work.[34] We hope that data presented in the current paper will help for future studies.

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