

High endothelial venules (HEVs): specialized endothelium for lymphocyte migration

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High endothelial venules (HEVs) are specialized postcapillary venules found in lymphoid tissues that support high levels of lymphocyte extravasation from the blood. Here, Jean-Philippe Girard and Timothy Springer highlight the unique properties of HEV endothelium, discuss the molecular mechanisms controlling HEV specialization and review evidence suggesting that HEVs could play an important role in the pathogenesis of chronic inflammatory diseases.

While patrolling the body in search of foreign antigen, lymphocytes continuously circulate from blood, through lymphoid and other tissues, and back through the lymphatics to the blood. This process, called lymphocyte recirculation, allows the dissemination of the immune response throughout the body, and thereby provides an effective immune surveillance for foreign invaders and alterations in the body's own cells^{1,2}.

The first critical step in lymphocyte migration from circulation into tissue is the adhesion of lymphocytes to vascular endothelium. In lymphoid organs, lymphocyte adherence and transendothelial migration occur at specialized postcapillary vascular sites called high endothelial venules (HEVs). Although HEVs are particularly abundant in the T-cell areas surrounding the B-cell follicles, they serve as the sites of entry both for T and B lymphocytes³. In humans, HEVs are found in all secondary lymphoid organs (with the exception of spleen, where lymphocyte emigration occurs via the blood sinusoids in the marginal zone), including hundreds of lymph nodes dispersed in the body, tonsils and adenoids in the pharynx, Peyer's patches (PPs) in the small intestine, appendix, and small aggregates of lymphoid tissue in the stomach and large intestine. Moreover, HEV-like vessels are observed in chronically inflamed nonlymphoid tissues and are believed to support lymphocyte recruitment into these sites⁴. In contrast to the endothelial cells from other vessels, the high endothelial cells of HEVs have a distinctive appearance, express specialized ligands for lymphocytes and are able to support high levels of lymphocyte extravasation^{5,6}. While many reviews have discussed the molecular mechanisms of lymphocyte homing and lymphocyte-HEV interactions^{2,7-9}, most have focused on lymphocytes and the lymphocyte homing receptor L-selectin, and considerably less attention has been given to the HEV side of the interaction. The purpose of this review is to highlight the specialized features of the HEV endothelium that allow lymphocyte emigration in HEVs to be so efficient, and to discuss the role of the tissue environment in the control of HEV specialization.

The specialized endothelium of HEVs

Structural features of HEVs

The endothelial cells of HEVs have a 'plump', almost cuboidal, appearance very different from the flat morphology of endothelial cells that line other vessels, and are therefore called high endothelial cells by reference to their thickness (Fig. 1). Another characteristic of HEVs, revealed by light-microscopic examination, is the presence of a large number of lymphocytes within their walls. This illustrates the function of HEVs in lymphocyte recruitment, and explains why these vessels were implicated in lymphocyte traffic from the time of their initial description¹¹.

At the ultrastructural level, high endothelial cells are characterized by a prominent Golgi complex, abundant polyribosomes and rough endoplasmic reticulum. This reveals an intense biosynthetic activity not observed in flat endothelial cells^{6,12}. They also contain many membrane-bound vesicular structures, multivesicular bodies, Weibel-Palade bodies and a variety of dense bodies, indicating that they are involved in secretion¹². Another important feature of HEVs, revealed by ultrastructural studies, is the existence of discontinuous 'spot-welded' junctions between high endothelial cells⁷. These discontinuous junctions differ from the tight junctions that characterize capillary and arterial endothelium but are similar to the 'non-occluding' junctions found in other postcapillary venules⁷. This property of HEVs probably facilitates the passage of lymphocytes between adjacent high endothelial cells and is likely to be one of the factors allowing massive lymphocyte emigration in HEVs.

HEV-specific markers

Despite intensive efforts, very few HEV-specific markers have been described. The best HEV marker currently available is a carbohydrate epitope recognized by the monoclonal antibody (mAb) MECA-79 (Ref. 13), which stains all HEVs within lymphoid tissues and does not react with postcapillary venules or large vessels in spleen, thymus or nonlymphoid tissues^{13,14}. MECA-79 mAb inhibits lymphocyte emigration through HEVs into lymph nodes *in vivo* and

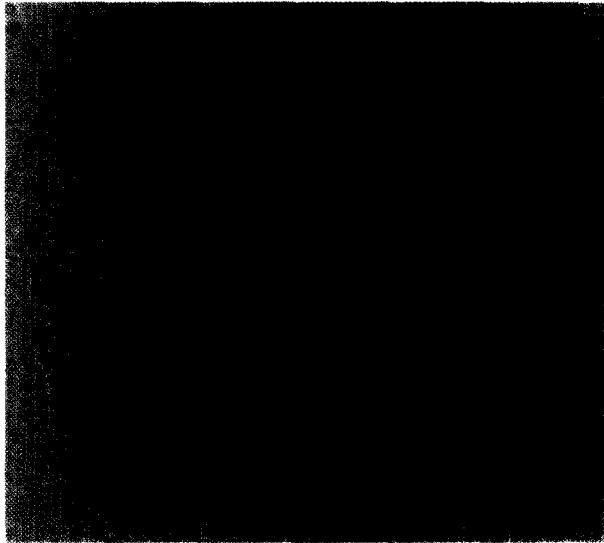


Fig. 1. High endothelial venule (HEV) in a human tonsil. The 'plump' high endothelial cells are stained by in situ hybridization with a digoxigenin-labeled cDNA probe encoding hevin, a secreted protein specifically expressed in HEVs from human tonsils¹⁰. Magnification = $\times 400$.

lymphocyte adhesion to lymph node and tonsil HEVs *in vitro*^{13,14}. Although initially produced against mouse HEVs, the mAb shows a wide crossreactivity among species¹⁵. The MECA-79 carbohydrate epitope decorates a family of HEV counter-receptors for L-selectin, both in mouse and human^{16,17}. Another mAb, HECA-452, recognizing a carbohydrate epitope expressed on human HEVs but not on other vessels, has been described. However, unlike MECA-79, this mAb is not HEV specific: HECA-452 recognizes a carbohydrate epitope related to the sialyl-Lewis x and sialyl-Lewis a (sLe^x and sLe^a) oligosaccharides and, in addition to reacting with high endothelium, crossreacts with monocytic cells, dendritic cells and a subset of skin-homing memory lymphocytes^{9,18}.

Two other HEV markers have been described in the mouse: (1) the mAb MECA-325 defines an antigen that can be induced in nonlymphoid endothelial cells by interferon γ (IFN- γ)¹⁹; and (2) the mAb MECA-367 recognizes mucosal addressin cell adhesion molecule 1 (MAdCAM-1)²⁰, a counter-receptor for L-selectin¹⁷ and $\alpha_4\beta_7$ integrin²¹ that is expressed in mucosal HEVs and in venules of intestinal lamina propria but can be induced in nonmucosal endothelial cells by tumor necrosis factor α (TNF- α) and IL-1 (Ref. 22).

HEV-specific O-linked glycosylation of L-selectin counter-receptors

Lymphocyte L-selectin plays a key role in the initial interaction of lymphocytes with HEVs *in vivo* by recognizing carbohydrate ligands on the following HEV glycoproteins: glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1)²³, CD34 (Refs 24,25) and MAdCAM-1 (Ref. 17). All of these glycoproteins contain highly rigid, extended, serine/threonine-rich, mucin-like domains that are densely substituted with O-linked oligosaccharides (Fig. 2). Tissue-specific O-linked glycosylation of these three mucin-like proteins is required for L-selectin binding. For example, GlyCAM-1 expressed in lactating mammary gland epithelial cells²⁸ or MAdCAM-1 expressed in cultured brain-derived

endothelial cells¹⁷ are not recognized by L-selectin. Similarly, CD34 is widely expressed in endothelial cells in most organs, but functions as an L-selectin counter-receptor only when appropriately decorated by specific oligosaccharides^{25,26}. Sialic acids are critical for recognition since sialidase treatment abolishes both L-selectin binding and lymphocyte adhesion to lymph node HEVs *in vitro* and *in vivo*^{16,29}. The sLe^x molecule has been proposed to function as an HEV ligand for L-selectin³⁰. However, sLe^x is expressed in endothelial cells that do not mediate lymphocyte recruitment *in vivo*³¹, thereby implying that the biological HEV ligands for L-selectin must be more complex than this simple tetrasaccharide. A 6'-sulfo-sLe^x isoform has recently been identified as a major capping group of GlyCAM-1 (Ref. 32) and sulfation of both GlyCAM1 (Ref. 33) and CD34 (Ref. 27) has been shown to be required for L-selectin recognition. Sulfated ligands for L-selectin have also been detected in rat lymph nodes³⁴.

Sulfation may be key to the uniqueness of the HEV ligands. Interestingly, both in humans and rats, the HEV endothelium has been shown to be unique amongst vascular endothelium by virtue of its capacity to incorporate large amounts of ³⁵SO₄ (Refs 4,35). The requirement for sulfate in high-affinity L-selectin ligands provides a functional basis for this metabolic specialization of the HEV endothelium. The critical role of sulfation in HEV is further reinforced by the fact that the only HEV-specific mAb currently available, MECA-79, is also sulfation dependent. Similar to L-selectin, MECA-79 recognizes sulfated oligosaccharides decorating GlyCAM-1 and CD34, and inhibition of sulfation of these glycoproteins abrogates recognition²⁷. The total absence of crossreactivity of MECA-79 with non-HEV cells of the human body¹⁴ suggests that the HEV-specific sulfated oligosaccharides recognized by MECA-79 and L-selectin are unique structures that are probably even more complex than 6'-sulfo-sLe^x. Clustering of oligosaccharides containing 6'-sulfo-sLe^x on mucin-like domains may be required for recognition since free oligosaccharides released from GlyCAM-1 do not bind with recognizable affinity to L-selectin³⁶, and all HEV counter-receptors for L-selectin identified to date contain highly O-glycosylated mucin-like domains. Recently, MECA-79⁻ ligands for L-selectin were identified on KG1a hematopoietic cells and neutrophils^{25,37,38}. CD34 from KG1a cells binds less well to L-selectin than CD34 isolated from tonsil stroma, perhaps reflecting an absence of sulfation²⁵.

Lymphocyte emigration in HEVs

Molecular mechanisms involved in lymphocyte migration through HEVs *in vivo*

Lymphocyte migration through HEVs *in vivo* is a very specific and efficient process. Lymphocytes circulating in the blood are able to discriminate between the HEV endothelium and the endothelium lining adjacent nonlymphoid tissues^{5,39}. Approximately 25% of lymphocytes circulating in HEVs will bind and emigrate^{39,40} and it has been estimated that this results in as many as 1.4×10^4 lymphocytes extravasating from the blood into a single lymph node (via HEVs) every second⁴¹. Although it was initially thought that a

single receptor-counter-receptor pair would explain the specificity of lymphocyte emigration in HEVs, it is now clear that the molecular mechanisms conferring this specificity are more complex, with many different molecules involved in a multistep process (Fig. 3; Ref. 8).

Functional inactivation of L-selectin by blocking antibodies^{42,43} or by gene knockout⁴⁴ results in a 99% decrease of lymphocyte migration to peripheral lymph nodes (PLNs) and a 50% reduction of lymphocyte emigration in PP HEVs. This latter result is consistent with the low but significant MECA-79 reactivity with PP HEVs (Ref. 45) and the expression in PP HEVs of a MECA-79⁺ subset of MAdCAM-1 molecules able to support L-selectin-mediated lymphocyte rolling *in vitro* (Ref. 17). Moreover, in sheep, pig and rabbit, staining of PP HEVs with MECA-79 is as intense as the staining of PLN HEVs, suggesting that, in some species, the requirement for L-selectin during lymphocyte emigration in PP HEVs could be even more critical¹⁵.

Studies with pertussis toxin, which is known to induce a profound lymphocytosis in humans and mice, have shown that a G-protein-mediated activation event is required for lymphocyte emigration in PLN and PP HEVs (Ref. 8). Recently, intravital microscopic studies of lymphocyte emigration in mouse PP HEVs have revealed that treatment with pertussis toxin has no effect on the initial 'rolling' interaction of lymphocytes with HEVs but inhibits an activation-dependent 'sticking' event required for lymphocyte arrest⁴⁰. This activation can be extraordinarily rapid since lymphocyte sticking can occur within 1–3 seconds after initiation of rolling³⁹. Although G-protein-coupled receptors for cytokines of the chemokine family have been proposed to play a role in this rapid activation of lymphocyte adhesiveness⁸, the local factors and molecules involved in physiological activation of lymphocytes in HEVs remain to be defined.

Two lymphocyte integrins involved in the activation-dependent sticking and arrest of lymphocytes in HEVs have now been characterized (Fig. 3). Antibodies against leukocyte function-associated molecule 1 (LFA-1) ($\alpha_L\beta_2$; CD11a/CD18) inhibit lymphocyte migration through PLN HEVs by 80% and through PP HEVs by 50% (Ref. 43), which is consistent with the constitutive expression of the LFA-1 counter-receptors intercellular adhesion molecule 1 (ICAM-1) and ICAM-2 on HEVs. Furthermore, antibodies against $\alpha_4\beta_7$ or its counter-receptor, MAdCAM-1, can inhibit up to 75% of lymphocyte emigration in PP HEVs (Refs 20,43). In addition to its role in lymphocyte arrest in PP HEVs, the $\alpha_4\beta_7$ -MAdCAM-1 pair could also mediate the initial recognition of PP HEVs by some lymphocytes, since $\alpha_4\beta_7$ has recently been shown to mediate L-selectin-independent attachment and rolling on MAdCAM-1 (Ref. 21). This is more likely to be true for lymphoblasts or memory lymphocytes expressing very high levels of $\alpha_4\beta_7$ than for naive lymphocytes, which are uniformly L-selectin^{hi} $\alpha_4\beta_7$ ^{lo} and probably absolutely require L-selectin for the initial recognition of PP HEVs. Interestingly, $\alpha_4\beta_7$, like L-selectin, is localized on the tips of lymphocyte microvilli, which mediate the initial interaction of lymphocytes with HEVs,

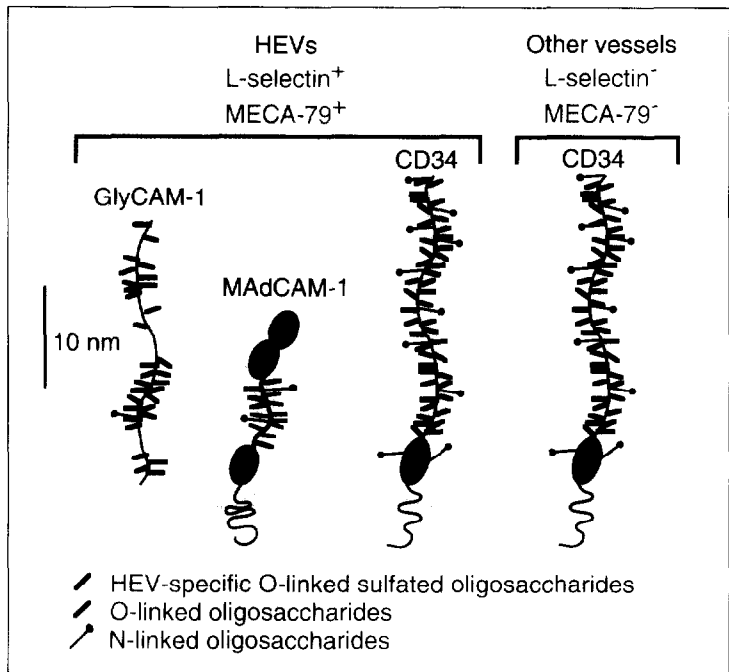


Fig. 2. High endothelial venule (HEV)-specific O-linked glycosylation of counter-receptors for L-selectin. Glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1)²³, mucosal addressin cell adhesion molecule 1 (MAdCAM-1)¹⁷ and CD34 (Refs 24,25) are HEV glycoproteins with mucin-like domains (containing a high percentage of serine and threonine residues) that present dense clusters of O-linked sulfated carbohydrates to lymphocyte L-selectin. Although CD34 is expressed in many other vessels in addition to HEVs (Ref. 26), it functions as a high-affinity L-selectin counter-receptor only when it is decorated by HEV-specific, sulfated, sialylated, fucosylated, O-linked oligosaccharides (shown in blue). Sulfation of CD34 is required for high-affinity L-selectin binding and recognition by the HEV-specific mAb MECA-79 (Refs 25,27). Modified figure reproduced with permission from Ref. 8.

and this unique topographical distribution is likely to explain why $\alpha_4\beta_7$, like L-selectin, is able to mediate lymphocyte attachment and rolling under flow²¹. The $\alpha_4\beta_1$ -vascular cell adhesion molecule 1 (VCAM-1) pair is important for lymphocyte emigration in inflammation but is not expected to play any role in lymphocyte binding to HEVs since VCAM-1 is not expressed on the latter (Refs 15,46; J-P. Girard and T.A. Springer, unpublished), except in rat where low levels have been detected⁴⁷.

The entire process of lymphocyte sticking to HEVs (rolling, activation, arrest) takes only a few seconds⁴⁰, while transendothelial migration and the passage of lymphocytes through the HEV basement membrane occur in approximately ten minutes⁴⁸. The molecular mechanisms involved in these two latter steps remain poorly characterized. However, modulation of lymphocyte adhesiveness is likely to be critical since migration of lymphocytes through HEV junctions and the basement membrane requires a succession of adhesive and de-adhesive states. Hevin, a recently identified major secreted component of human HEVs, homologous to the extracellular matrix (ECM) adhesion modulator SPARC (for 'secreted protein acidic and rich in cysteine')¹⁰, could be one of the factors modulating adhesiveness in HEVs and facilitating lymphocyte trans-endothelial migration.

Other molecules, such as the hyaluronan receptor CD44 (Ref. 49) or the vascular adhesion protein VAP-1

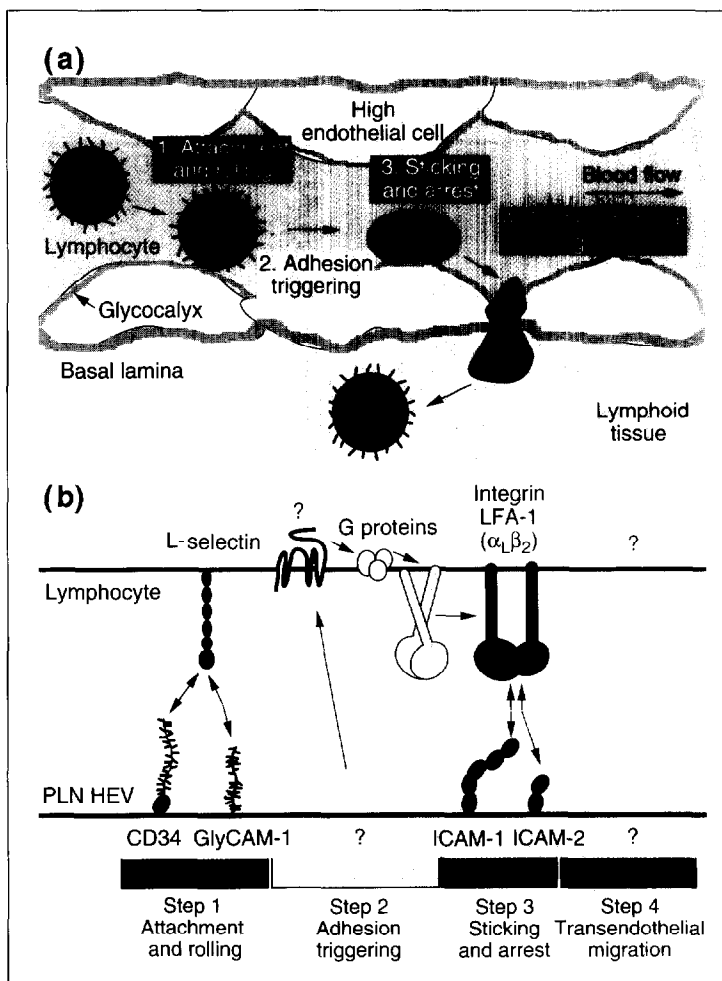


Fig. 3. Lymphocyte emigration in high endothelial venules (HEVs). (a) Lymphocytes circulating in the blood initiate contact with the high endothelial cells via microvilli. This initial adhesion is transient and is often manifested in rolling of the interacting cells along the HEV endothelium (step 1: attachment and rolling). Activation of lymphocyte adhesiveness (step 2: adhesion triggering) results in firm attachment, which becomes stable to physiological shear force (step 3: sticking and arrest). Lymphocytes then migrate through endothelial cell junctions and enter lymphoid tissue after crossing the HEV basal lamina (stage 4: transendothelial migration). The luminal surface of HEVs is coated by a prominent glycocalyx and this may play an important role in lymphocyte emigration (see text for details). (b) The initial interaction of lymphocytes with HEVs in peripheral lymph nodes (PLNs) is mediated by L-selectin, which recognizes the following HEV mucin-like counter-receptors: CD34 and glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1). Activation of lymphocyte adhesiveness occurs via G-protein-coupled receptors, but the local factors involved in physiological activation of lymphocytes in HEVs are not yet characterized. The integrin leukocyte function-associated molecule 1 (LFA-1) ($\alpha_L\beta_2$; CD11a/CD18) and its HEV counter-receptors intercellular adhesion molecule 1 (ICAM-1) and ICAM-2 play a major role in lymphocyte arrest in PLN HEVs. The molecular mechanisms involved in lymphocyte transendothelial migration remain poorly characterized. Modified figure reproduced with permission from Ref. 8.

(Ref. 50), have been proposed to play a role in lymphocyte emigration in HEVs, based on results of *in vitro* assays of lymphocyte adhesion to HEVs. However, a recent report showing that CD44 is not required for lymphocyte recirculation through murine HEVs *in vivo* casts some doubts upon the role of CD44 as a major lymphocyte receptor for HEVs (Ref. 51). Therefore, confirmation of a role in lymphocyte emigration in HEVs for CD44, VAP-1, or other molecules identified using *in vitro* assays, will require further *in vivo* studies.

A role for the HEV glycocalyx in lymphocyte emigration?

Unlike capillaries and other vessels, the luminal surface of HEVs is coated by a prominent glycocalyx⁶. By facilitating the retention (immobilization) of secreted molecules on the luminal surface of HEVs, this thick glycocalyx could play an important role in lymphocyte emigration. For example, the HEV glycocalyx has been proposed to be involved not only in the retention of the L-selectin ligand GlyCAM-1, which is heavily sulfated and secreted by HEVs, but also in the immobilization of the soluble factors predicted to activate lymphocyte adhesiveness in HEVs (Ref. 7). Other proteins secreted by HEVs, such as the ECM proteins laminin and fibronectin, also seem to be retained in the HEV glycocalyx. Immunohistochemical studies have revealed that, while laminin and fibronectin are only detected in the basement membrane of vessels lined by flat endothelial cells, they can be found both at the basal and luminal surface of HEVs (Refs 52,53; J-P. Girard and T.A. Springer, unpublished). Interestingly, antibodies against laminin have been reported to reduce lymphocyte emigration into PLNs *in vivo*⁵⁴. Moreover, antibodies against either fibronectin or the fibronectin receptor, very late antigen 5 (VLA-5), have been found to inhibit significantly the *in vitro* adhesion of lymphocytes to HEVs in frozen sections of human lymph nodes⁵³ and the binding of human lymphocytes to cultured rat high endothelial cells⁵⁵. Therefore, the integrins VLA-5 ($\alpha_5\beta_1$) and VLA-6 ($\alpha_6\beta_1$), which are constitutively expressed on recirculating lymphocytes, and their ECM ligands, fibronectin and laminin, which appear to be retained in the glycocalyx on the luminal surface of HEVs, could play an accessory role in lymphocyte arrest in HEVs.

Control of HEV specialization by the local microenvironment

Components of afferent lymph regulate HEV specialization

HEVs represent one of the most striking examples of endothelial differentiation. It appears that all of the specialized features of HEVs are under the control of the local tissue environment. Indeed, when PLNs are deprived of afferent lymph, HEVs convert from a high-to a flat-walled endothelial morphology and lose their ability to support lymphocyte traffic^{56,57}. More recently, it was shown that ligation of afferent lymphatics also results in a loss of the luminal staining of HEVs with MECA-79, a tenfold reduction in ³⁵S₄ incorporation, a profound downregulation of GlyCAM-1 mRNA levels and a complete inhibition of L-selectin-mediated adhesion to HEVs (Refs 58,59). These studies suggest that lymph contains cells or factors that regulate HEV phenotype and function. One of these factors could be antigen draining into the lymph from peripheral tissues, since the lack of antigenic stimulation and subsequent activation of the PLNs may result in the absence of cytokines required for the induction of HEV characteristics (see below). Consistent with this hypothesis, direct injection of antigen into the node has been shown to restore the typical HEV morphology in PLNs deprived of afferent lymph⁵⁶.

The fact that lymph components are required to maintain the HEV phenotype illustrates perfectly the

critical role of the lymphoid tissue microenvironment in the control of HEV specialization. It also provides an explanation for the finding that, although high endothelial cells have been maintained in *in vitro* cultures⁶⁰, there has been no report so far that these cells express the L-selectin counter-receptors GlyCAM-1, MAdCAM-1 and CD34. By contrast, a recent study has shown that high endothelial cells rapidly lose the HEV-specific marker MECA-325 when they are grown *in vitro*⁶¹.

Comparison of HEV and blood-brain barrier (BBB) endothelium

Another striking example of modulation of endothelial specialization by the local tissue environment is provided by the endothelium of the blood-brain barrier (BBB)⁶². Special features of this endothelium include the presence of tight junctions with very high electrical resistance between adjacent endothelial cells and the expression of specific markers such as HT7/neurothelin, a member of the immunoglobulin superfamily (Fig. 4). The ability of brain endothelial cells to form a BBB is not intrinsic to these cells but, rather, is induced by the brain environment: elegant chick-quail chimera experiments have revealed that endothelial cells from brain capillaries that are permitted to vascularize peripheral tissues become leaky⁶³; conversely, endothelial cells originally derived from peripheral capillaries form a competent BBB (Ref. 63) and express HT7/neurothelin⁶⁴ when they vascularize brain tissue. Similarly, transplantation of small lymph node fragments into kidneys has revealed that capillaries originally derived from the renal parenchyma invade the residual autograft and differentiate into HEVs (Ref. 65). These experiments suggest that morphologically and functionally distinct endothelial cell types are not predetermined by lineage, and that HEV and BBB endothelial cells may differentiate under influences from their local microenvironments.

In the brain, the cells that are responsible for inducing endothelial cells to form the tight junctions characteristic of the BBB have been identified. Astrocytes, a distinct type of glial cell that surround endothelial cells and contact the basal lamina via end-feet processes, have been shown to induce BBB properties in non-neural endothelial cells *in vivo*⁶⁶. Astrocyte-conditioned medium was found to be sufficient to induce BBB characteristics, including the expression of HT7/neurothelin⁶². These results emphasize the critical role of the brain microenvironment (astrocytes are the nearest neighbors of brain capillaries) in the control of the specialization of the BBB endothelium.

In the case of HEVs, the cell types involved in the induction and maintenance of the specialized phenotype have not yet been identified. Since subcapsular sinus macrophages and interdigitating dendritic cells (IDCs) are depleted from lymph nodes after the occlusion of afferent lymphatics, these cells have been proposed to play a role in the control of the differentiation of the HEV endothelium⁵⁶⁻⁵⁸. However, when the same macrophage population that is affected by de-afferentiation was depleted specifically, no effects on the capacity of HEVs to bind lymphocytes were observed⁶¹. Moreover,

when the effects of afferent lymphatic occlusion were reversed, and HEVs had regained their characteristic morphology and phenotype, this population of macrophage was not replaced, indicating these macrophages are not required for HEV differentiation⁵⁸. By contrast, arguments in favor of a role of IDCs in HEV differentiation are very strong. After reversal of occlusion, the restoration of HEV phenotype and function correlates with the reappearance of IDCs (Ref. 58). Study of HEV development in lymph node autografts revealed that the first HEVs appeared after the few surviving IDCs of the graft showed marked proliferation and produced amorphous substances, which may induce the development of HEVs from kidney capillaries⁶⁵. Similarly, during the development of the human immune system, the appearance of HECA-452⁺ IDCs in the lymph nodes, tonsils and appendix always precedes the development of HEVs (Ref. 67). Finally, in chronic inflammatory diseases such as rheumatoid arthritis or autoimmune thyroiditis, the development of HEVs is also preceded by the infiltration of HECA-452⁻ IDCs (Refs 68,69). These studies, together with the observation that ultraviolet irradiation (to which dendritic cells are very sensitive) can greatly reduce lymphocyte migration through PLN HEVs (Ref. 57), support the notion that IDCs play a key role in the induction and maintenance of HEV characteristics (Fig. 4).

Since HEVs are always associated with dense lymphocytic infiltration in peripheral lymphoid organs or sites of chronic inflammation, and the development of HEVs parallels a large-scale influx of lymphocytes, it has been suggested that recirculating lymphocytes may be required for HEV differentiation. However, studies of HEV development in athymic or nude mice have revealed that differentiation of the HEV endothelium is independent of the concentration of lymphocytes in the blood⁷⁰. Moreover, the loss of HEV characteristics in lymph nodes deprived of afferent lymphatics, despite normal numbers of recirculating lymphocytes in the blood, clearly shows that recirculating lymphocytes are not sufficient to induce the HEV phenotype⁵⁶. Furthermore, recent studies suggest that lymphocytes are probably not required for induction of the HEV phenotype: MAdCAM-1 and MECA-79 are induced on HEV-like vessels in the pancreas of IFN- γ -transgenic severe combined immunodeficiency (SCID) mice, which are deficient in mature T and B lymphocytes⁷¹; and normal HEVs are present in patients with complete DiGeorge syndrome, despite these individuals being athymic and devoid of T cells⁶⁷. Therefore, lymphocytes are probably not directly involved in the induction of HEV differentiation and are more likely to play an indirect role by allowing a certain level of immune activity required for the maintenance of the specialized HEV phenotype.

A role for cytokines and ECM proteins in induction and maintenance of HEV specialization?

One major difference in the microenvironment of HEVs and nonlymphoid postcapillary venules is the presence around HEVs of many cytokines generated in lymphoid organs during activation of lymphoid cells. Therefore, it is not surprising that cytokines appear to

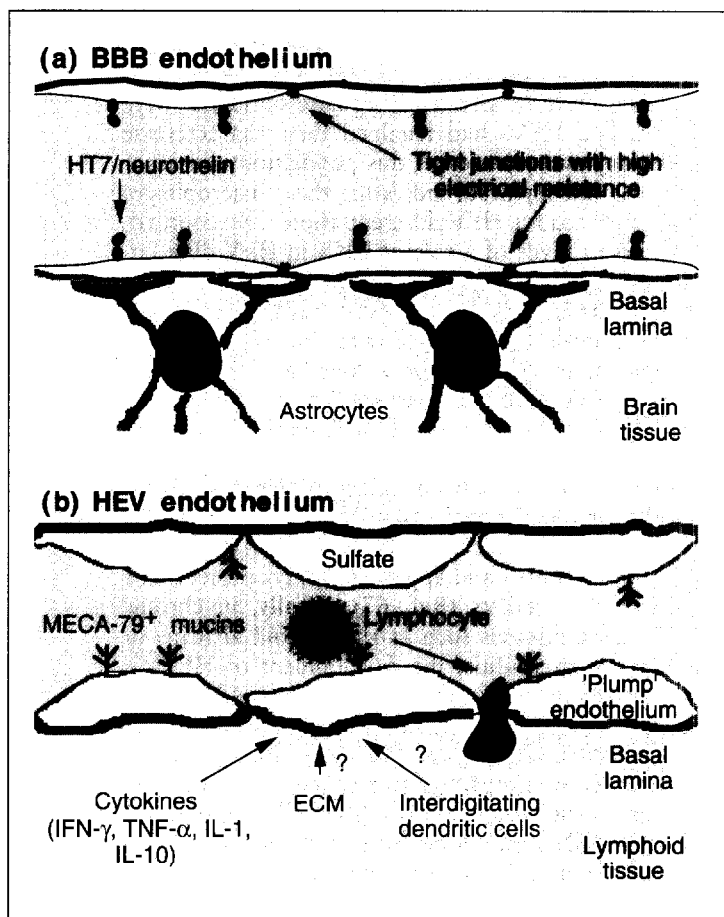


Fig. 4. Control of endothelial specialization by the tissue environment. (a) In the brain, vessels forming the blood-brain barrier (BBB) are characterized by the presence of tight junctions with high electrical resistance and the expression of the specific marker HT7/neurothelin. Astrocytes, which contact the basal lamina of these vessels via their end-feet processes, are able to induce these two BBB characteristics in non-neural endothelial cells *in vivo*. (b) In lymphoid tissues, the endothelial cells of high endothelial venules (HEVs) have a 'plump' morphology, incorporate high levels of sulfate, express specific ligands for lymphocytes defined by the HEV-specific monoclonal antibody (mAb) MECA-79 and are able to support high levels of lymphocyte extravasation. All of these characteristics are under the control of the lymphoid tissue environment. Although the factors and cell types involved in the induction and maintenance of the specialized HEV phenotype are not well characterized, the important components appear to be cytokines produced locally or supplied by afferent lymph, extracellular matrix (ECM) proteins surrounding the high endothelial cells and interdigitating dendritic cells.

Abbreviations: IFN- γ , interferon γ ; IL-1, interleukin 1; TNF- α , tumor necrosis factor α .

play a critical role in the induction and maintenance of the HEV phenotype. In mice, MECA-325 and MAdCAM-1 have been shown to be induced in cultured nonlymphoid endothelial cells by IFN- γ (Ref. 19) and TNF- α or IL-1 (Ref. 22), respectively, providing evidence that the specialized phenotype of HEVs could be controlled by local factors associated with immune activity. This possibility is further reinforced by *in vivo* studies in transgenic mice, which have revealed that MAdCAM-1 and, subsequently, MECA-79 are induced on HEV-like vessels in the pancreas of mice expressing IFN- γ or IL-10 in pancreatic β cells^{71,72}. Together, these studies suggest that cytokines associated with the immune response play a key role in the induction and maintenance of the specialized HEV phenotype.

However, cytokines are probably not sufficient by themselves to induce HEV specialization, and it is

likely that other microenvironmental factors, particularly ECM molecules, are also involved. The composition of the ECM in the vicinity of the high endothelial cells has been proposed to influence the permanent HEV phenotype in particular lymphoid organs. This hypothesis is based on the observation that transplantation of adult PLNs into a mucosal site, or mesenteric lymph nodes into a peripheral site, did not result in any change of the HEV phenotype, despite probable differences in cytokine and cellular composition of lymph nodes and afferent lymph at mucosal and peripheral sites⁷³. It is well known that, *in vitro*, differentiation of endothelial cells can be directed by the ECM on which the cells are grown⁷⁴. The ECM plays a crucial role in functional differentiation and cell-type-specific gene expression⁷⁵ and, in many situations, ECM-induced cell differentiation is reflected in changes in cell shape. For example, ECM induces mammary epithelial cells to adopt a highly columnar morphology required for vectorial secretion of milk and, together with lactogenic hormones, plays a critical role in the induction and maintenance of the differentiated phenotype of these epithelial cells⁷⁵. Strikingly, expression of the L-selectin counter-receptor GlyCAM-1, which is generally restricted to the plump endothelial cells of HEVs in lymph nodes, is induced in these columnar epithelial cells during lactation²⁸. Therefore, it is tempting to suggest that expression of GlyCAM-1 in the highly differentiated endothelial cells of HEVs could also require the cooperation of two signals, one originating from ECM proteins and the other from cytokines supplied by afferent lymph or generated locally. ECM proteins important for induction of the plump morphology and specialized phenotype of HEV cells could comprise widespread components, such as laminin, that play a key role in differentiation of mammary epithelial cells⁷⁵, or proteins with more-restricted expression such as the recently identified SPARC-like protein hevin¹⁰. Interestingly, hevin has recently been found to induce a rounded, plump morphology in endothelial cells cultured *in vitro* (J-P. Girard and T.A. Springer, unpublished). However, confirmation of a role for hevin, or other ECM proteins, in the differentiation of the HEV endothelium will require further studies.

HEVs in chronic inflammatory diseases

HEVs in extralymphoid sites of chronic inflammation in rodents

In the nonobese diabetic (NOD) mouse, which is a model for human insulin-dependent diabetes mellitus (IDDM), vessels with HEV characteristics (e.g. plump endothelial cells, numerous lymphocytes in the vessel walls) are observed during inflammation of the pancreas. Expression of MECA-79 and MECA-367 (MAdCAM-1) is induced on these HEV-like vessels^{76,77} during the development of insulinitis, whereby lymphocytes infiltrate the pancreatic islets. Staining with MECA-79 is consistent with the induction of functional L-selectin ligands, CD34, MAdCAM-1 and GlyCAM-1 (Ref. 26). The induction of GlyCAM-1 in the inflamed pancreas of NOD mice is particularly striking since GlyCAM-1 expression in mice had previously been shown to be restricted to PLN and mesenteric lymph node (MLN) HEVs (Ref. 23)

Table 1. High endothelial venules (HEVs) in chronic inflammatory diseases*

Disease ^b	Target organ	Plump endothelium	HEV marker MECA-79	HEV marker HECA-452
Rheumatoid arthritis	Synovium	+	+	+
Crohn's disease	Gut	+	?	+
Ulcerative colitis	Gut	+	?	+
Graves' disease	Thyroid	+	+	+
Hashimoto's thyroiditis	Thyroid	+	+	+
Diabetes mellitus	Pancreas	+	+	NA ^c

*See text for Refs.

^bData derived from human chronic inflammatory diseases [except the data for diabetes, which were derived from nonobese diabetic (NOD) mice].

^cNot applicable.

Together, these results indicate that HEV-like vessels induced by chronic inflammation in extralymphoid sites appear to be phenotypically similar to HEVs from lymphoid tissues. The induction of MECA-79 and MAdCAM-1 on the endothelium correlates with the expression of their counter-receptors L-selectin and $\alpha_4\beta_7$ on cells infiltrating the islets⁷⁶. Recent *in vivo* studies have revealed that these two receptor-counter-receptor pairs, $\alpha_4\beta_7$ -MAdCAM-1 and L-selectin-MECA-79, play a major role in the recruitment of lymphocytes from blood into the inflamed pancreas⁷⁸. Treatment of NOD mice with function-blocking mAbs specific for L-selectin and α_4 integrins resulted in the inhibition of insulinitis and the prevention of autoimmune diabetes.

In addition to pancreatic lesions, NOD mice also display inflammatory infiltration of the salivary glands, and HEV-like vessels expressing MECA-79 but not MAdCAM-1 were observed in areas of dense lymphocytic infiltration⁷⁷. Conversely, MAdCAM-1 but not MECA-79 has been found to be induced in brain endothelial cells during chronic relapsing experimental allergic encephalomyelitis (EAE) in Biozzi mouse⁷⁹. The differential expression of MECA-79 and MAdCAM-1 in HEV-like vessels from different sites suggests that the microenvironment in extralymphoid tissues directly influences the phenotype of the HEVs that are induced in chronic inflammatory conditions.

HEVs in human chronic inflammatory diseases

Vessels with HEV characteristics appear in human tissue in association with long-standing chronic inflammation (Table 1). Such vessels exhibit plump endothelial cells, take up and incorporate high levels of $^{35}\text{SO}_4$, contain many luminal and intramural lymphocytes (presumably in the process of extravasating) and mediate *in vitro* lymphocyte adhesion⁴.

In rheumatoid arthritis, it has been observed that the level of sulfate incorporation as well as the 'plumpness' (or 'tallness') of the endothelium in areas of lymphocytic infiltration in the synovial membrane are closely related to the concentration of the lymphocytes in the perivascular infiltrates⁸⁰. Similarly, expression of

MECA-79 and HECA-452 on these vessels is most pronounced in association with extensive lymphoid infiltrates^{14,69}. Therefore, the development of bona fide HEVs in the synovial membrane of patients with rheumatoid arthritis is likely to facilitate large-scale influx of lymphocytes, leading to amplification and maintenance of chronic inflammation. Although a distinct endothelial cell recognition system has been proposed to control lymphocyte emigration into inflamed synovium⁸¹, recent studies support the participation of several known adhesion molecules (L-selectin, β_1 , β_2 and α_4 integrins) acting in sequence, rather than a single synovial receptor-counter-receptor pair^{53,82}.

The development of HEVs after prolonged inflammatory stimulus is not restricted to diseased synovium, but can also occur in other tissues, particularly the gut and thyroid. During chronic inflammation of the gut in inflammatory bowel diseases (Crohn's disease and ulcerative colitis) or the thyroid in autoimmune thyroiditis (Graves' disease and Hashimoto's thyroiditis), areas of dense lymphocytic infiltration contain vessels with plump endothelium expressing MECA-79 and HECA-452 (Refs 14,18,68,83). These observations suggest that HEVs could play an important role in the pathogenesis of these diseases by mediating abnormal lymphocyte recruitment to the gut or the thyroid. MECA-79⁺ venules with plump endothelium have also been detected in other sites of chronic inflammation, including many cutaneous inflammatory lesions¹⁴. The presence of MECA-79⁺ HEV-like vessels in many different human chronic inflammatory diseases indicates that L-selectin is likely to play a major role in lymphocyte emigration at chronic inflammatory sites.

Concluding remarks

The HEV endothelium is unique amongst vascular endothelium by virtue of its capacity to recruit large numbers of lymphocytes. In the human body, it is estimated that as many as 5×10^6 lymphocytes extravasate from the blood through HEVs every second. The specificity and efficiency of this process is explained by specialized features of the HEV endothelium, such as

the expression of mucin-like glycoproteins decorated with HEV-specific oligosaccharides. In the future, it will be important to define precisely the molecular mechanisms involved in the induction and maintenance of the specialized HEV phenotype. HEV-like vessels induced by chronic inflammation in extralymphoid sites appear to be phenotypically and functionally similar to HEVs from lymphoid tissues. Thus, a better understanding of the mechanisms controlling development and maintenance of HEVs could provide the basis of novel therapeutic approaches for the treatment of human chronic inflammatory diseases, including rheumatoid arthritis and inflammatory bowel diseases, in which HEV-like vessels facilitate large-scale influx of lymphocytes, leading to amplification and maintenance of chronic inflammation.

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letters

Heterogeneity of organ-specific autoimmune diseases

In a recent article in *Immunology Today*¹, Liblau et al. reviewed the role of CD4⁺ T helper 1 (Th1) and Th2 cells in the pathogenesis of organ-specific autoimmune diseases. Insulin-dependent diabetes mellitus (IDDM) and experimental autoimmune encephalitis (EAE) were discussed as examples of Th1-mediated organ-specific damage. They state that 'similar data exist for other autoimmune diseases'. In addition, they suggest that immunization with autoantigens in the presence of interleukin 4 (IL-4) may provide an intervention strategy in such diseases by diverting the immune response away from the Th1 cells.

This review is representative of the common implication that organ-specific autoimmune diseases are T-cell mediated. In fact, some hu-

man organ-specific autoimmune diseases are caused directly by autoantibodies. Among these are Graves' disease (autoantibodies to the thyroid-stimulating hormone receptor) and myasthenia gravis (autoantibodies to the acetylcholine receptor) (reviewed in Refs 2,3, respectively). Indeed, the incidence of Graves' disease in the general population is tenfold higher than that of IDDM (~100 versus 10 cases per 100 000 per year)^{4,5}.

Clearly, CD4⁺ T cells are involved in autoantibody generation. However, in a disease such as Graves' disease, boosting the Th2 response is likely to be inappropriate therapy. Conversely, diverting the Th2 to a Th1 response could lead to thyroiditis and hypothyroidism, a form of autoimmune thyroid disease that is even more common than Graves' disease. We wish to make a plea that human organ-specific autoimmune disease be perceived as heterogeneous, and not as a monolithic group of diseases directly caused by T cells.

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