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Review Article

Role of high endothelial postcapillary venules and selected adhesion molecules in periodontal diseases: a review

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Periodontitis is accompanied by the proliferation of small blood vessels in the gingival *lamina propria*. Specialized postcapillary venules, termed periodontal high endothelial-like venules, are also present, and demonstrate morphological and functional traits similar to those of high endothelial venules (HEVs) in lymphatic organs. The suggested role of HEVs in the pathogenesis of chronic periodontitis involves participation in leukocyte transendothelial migration and therefore proinflammatory effects appear. Recent observations suggest that chronic periodontitis is an independent risk factor for systemic vascular disease and may result in stimulation of the synthesis of acute phase protein by cytokines released by periodontal high endothelial cells (HECs). However, tissue expression of HEV-linked adhesion molecules has not been evaluated in the gingiva of patients with chronic periodontitis. This is significant in relation to potential therapy targeting expression of the adhesion molecules. In this review, current knowledge of HEV structure and the related expression of four surface adhesion molecules of HECs [CD34, platelet endothelial cell adhesion molecule 1, endoglin and intercellular adhesion molecule 1 (ICAM-1)], involved in the key steps of the adhesion cascade in periodontal diseases, are discussed. Most studies on the expression of adhesion molecules in the development and progression of periodontal diseases pertain to ICAM-1 (CD54). Studies by the authors demonstrated quantitatively similar expression of three of four selected surface markers in gingival HEVs of patients with chronic periodontitis and in HEVs of reactive lymph nodes, confirming morphological and functional similarity of HEVs in pathologically altered tissues with those in lymphoid tissues.

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Histology of high endothelial venules

High endothelial venules were originally described in 1898 by Thome (1). At that time they were already linked to the migration of lymphocytes. In 1964, in the rat model, Marchesi & Gowans (2) discovered that the passage of lymphocytes from the circulation to the lymph nodes took place in HEVs located in the paracortex of lymph nodes. These investigators established that lymphocyte migration across HEVs is a physiological process (3).

HEVs are composed of high endothelial cells (HECs), of a cuboid or a cylindrical shape, containing a ground glass cytoplasm. The structure of HECs is determined primarily by the presence of numerous and well-developed subcellular organelles (4–6).

HEVs have the general appearance of venules, vary from 20 to 100 μm in diameter and are lined by between three and 10 cells when viewed in transverse section (5) (Fig. 1A).

Detailed studies document that the tall and plump appearance of HECs reflects constitutive surface expression of multiple adhesion molecules and chemokines (7,8). The nuclei in HECs are large and irregular with scattered chromatin and a centrally located nucleolus (9). A well-developed Golgi apparatus is accompanied by numerous small transport vesicles (7,10,11). The cytoplasm is rich in microfilaments and contains numerous mitochondria of variable shape and size. Further characteristics include a well-developed rough endoplasmic reticulum (4,5) and numerous polyribosomes (6). Significant numbers of Weibel–Palade bodies and other vesicular structures of a high density point to a high secretory potential of HECs (6). The cell membrane demonstrates irregularities, encompassing numerous cellular processes, promoting the potential for lymphocyte trapping (8,11). An additional feature of the surface is a thick layer of glycocalyx (4,6). The basement membrane abbuting the HECs is well developed but manifests as a loose irregular multilayer (11). Some authors have described a very thick basement membrane of lymph nodes in HEVs (10). Adjacent pericytes have distinguishing cytoplasm containing numerous mitochondria and myofilaments (4). Tight intercellular junctions in the endothelium of HEVs are poorly developed (4). The luminal surface of HEVs presents a rich expression of ligands, components of the peripheral node addressins group, which play a fundamental role during the adhesive cascade, in the activation and passage of lymphocytes to the extravascular compartment (12,13). The typical spongy morphology is induced by hevin and trombospondin-1, as confirmed by *in vitro* studies (6,14).

A typical trait of HEVs is the association with high numbers of small, mature lymphocytes (5). Some of the lymphocytes are also observed to be embedded within the HECs (8,10). Lymphocytes form characteristic concentric circles around HEVs (15). At present it is known that it is mainly T and B lymphocytes that migrate through the HEVs of peripheral lymphatic organs while all types of leukocyte may pass through the walls of postcapillary venules in other organs (6).

HEVs are present in all peripheral lymphatic organs, such as lymph nodes, tonsils, Peyer's patches in small intestine and the appendix, or in small accumulations of lymphoid tissue in the stomach, the small intestine and the respiratory tract (6,8,9,16), but they are absent from the spleen (9). Individual HEVs were noted in thymic medulla (9).

HEVs are important for defense against pathogens (8,15,17). The principal physiological function of HEVs is their participation in the migration and recirculation of T and B lymphocytes in lymphoid organs. This allows for the early recognition of foreign antigens present on mature dendritic cells and for the initiation of immune responses by the production of effector cells and memory cells (18,19). Recognition of the vessel wall and slow rolling (step 1) of lymphocytes along the endothelial cells (ECs) (8,20) is followed by the triggering of pertussis toxin-sensitive G-protein-coupled receptors by chemoattractants (step 2) (20). Tight adhesion (step 3) precedes transmigration (diapedesis) of lymphocytes (step 4).

Secondary lymphoid-tissue chemokine was the first chemokine discovered with a specific attraction for naïve T lymphocytes; this promoted homing of these cells in secondary lymphoid organs (21,22). The expression of secondary lymphoid-tissue chemokine by HEVs in Peyer's patches and the presence of a receptor for secondary lymphoid-tissue chemokine, the CC chemokine receptor 7, on T lymphocytes provide key mechanisms for discriminating between T and B lymphocytes (22).

In the transmigration of lymphocytes a significant role is fulfilled by adhesion molecules – both those present on the surface of lymphocytes (the so-called homing receptors) and those present on HEVs (23). The first group includes leukocytic (L)-selectins and the second group contains the complex of glycoprotein ligands belonging to the group of vascular addressins (24,25). The addressins include glycosylation-

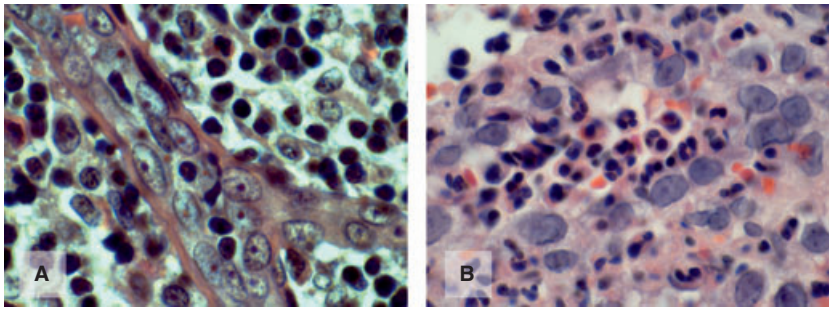

Fig. 1. Fragment of reactive lymph node (A) and gingiva of patients with chronic periodontitis (B) with postcapillary high endothelial venules (HEVs). Note the specialized high (cuboidal) endothelial cells (HECs), containing a ground glass cytoplasm. The HECs are large and bulge prominently into the vessel lumen. The nuclei are large with prominent nucleoli. Polymorphonuclear leukocytes (PMNs) constitute the majority of leukocytes in the lumen of periodontal HECs (B). Hematoxylin and eosin staining. Objective magnification, ×100.

dependent cell adhesion molecule-1(GlyCAM-1), CD34, mucosal adressin cell adhesion molecule-1 (MAdCAM-1), podocalyxin and endomucin (26–29). The recently discovered sialomucins of the addressin group include nepmucin, which is present on the microvilli of lymph-node HECs but absent from Peyer's patch HECs. Nepmucin is proposed to promote both the stage of rolling of lymphocytes on the HEV wall and the stage of strict adherence of lymphocytes to the vascular wall (25). Motility of lymphocytes and migration between the ECs is promoted also by the anti-adhesive glycoprotein, hevin, discovered in HEVs from human lymphatic organs (6). The above-mentioned proteins, produced in large quantities by HECs, are described as immunocytochemical markers of HEV-type blood vessels. Studies by Liao & Ruddle (30) confirmed earlier observations that normal function of HEVs and the so-called plasticity and remodeling of blood vessels requires the presence of functional lymphatic vessels in lymph nodes. The process of cell passage through HEV endothelium involves not just adhesion molecules but also appropriate enzymes. Most investigations have focussed on the role of fucosylotransferases (FucT-IV and FucT-VII) and a sulfotransferase (termed HEC-GlcNAc6ST) that also fulfil a function as ligands for L-selectin (24,31–33). Involvement of metalloproteinases was also established in the transmigration of lymphocytes through HEV and the HEV basement membrane (18).

HEVs can appear also in extralymphoid organs, embroiled in a chronic inflammatory process (34). In contrast to HEVs of lymphoid organs, the blood vessels are termed, by some investigators, as HEV-like venules (5,34). They are present in particular in regions of marked accumulation of lymphocytes (5,9,34,35).

In contrast to the succesful identification of adhesion molecules and the receptors controlling tissue-specific lymphocyte trafficking to lymphoid organs and some nonlymphoid tissues (e.g. skin and gut), relatively little is known about the expression and the mechanisms regulating trafficking to the other nonlymphoid tissues (20,23).

Four molecularly distinct adhesion and signaling events in leukocyte recirculation between the blood and lymphoid/nonlymphoid tissues are demonstrated schematically in Fig. 2.

Morphogenesis of HEVs

There remains a paucity of data on the mechanisms of HEV formation in lymphoid tissues and in sites of chronic inflammation (20). Probably, mature blood vessels differentiate from primitive vessels in response to a stable, slow blood flow at low pressure (6,36). HECs may prove to be an ontogenetically separate cell line. Alternatively, they could be induced by local factors and therefore represent the reversible phenotype of a flat endothelium. While the former hypothesis cannot be excluded, it remains possible that microenvironmental activity may evoke alterations in the HEC phenotype (6,9,16,37). Control of a specialized phenotype in venous blood vessels was suggested to include the action of a nuclear factor, typical for HEVs (NF-HEV) (38). Moreover, Hendriks & Eestermans (39) established that in lymph nodes with no lymph inflow, HEVs may change to postcapillary venules lined with a flat endothelium, which is unable to trap lymphocytes. Other authors demonstrated a rapid and striking loss of morphological and functional traits of human HEVs following isolation from lymphoid organs, that is, following removal from a special microenvironment. These studies demonstrated complete loss of at least two HEV markers – Duffy antigens for chemokines and the HEV-specific fucosylotransferase (Fuc-TVII) – as well as decreased expression of many other genes (37).

HEVs and systemic diseases

Most frequently, HEVs are present in the mucosa of various organs involved in chronic inflammatory or neoplastic processes. The number of HEVs increases in regions with elevated numbers of inflammatory cells, particularly T and B lymphocytes (5,21,22,34,35). Under the stimulus of a local immune reaction or persisting chronic inflammation, postcapillary venules may transform into HEVs to facilitate leukocyte migration into the affected tissues (9,40).

Accordingly, the presence of HEVs was demonstrated in a variety of disease states, including arthritis, ulcerative colitis, Crohn's disease,

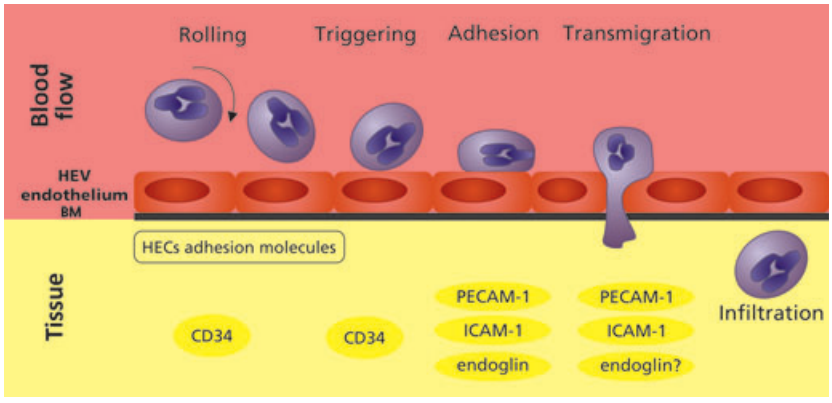

Fig. 2. Multiple stages of leukocyte (mainly lymphocytes) transendothelial migration. The cascade begins with lymphocyte rolling (step 1). Then, lymphocyte adhesion triggers pertussis toxin-sensitive G-protein-coupled receptors by chemoattractants (step 2). Chemoattractant binding, in turn, induces intracellular signals for firm adhesion of lymphocytes (step 3). Finally, lymphocytes transmigrate across the high endothelial cells (HECs) (step 4), penetrate the underlying basement membrane and infiltrate the parenchyma of the tissue. Transmigration of leukocytes occurs through a stepwise interaction with adhesion molecules expressed on the luminal surface of postcapillary high endothelial venules (HEVs) (see Fig. 3) and their receptors on leukocytes [according (20), modified].

Hashimoto-type thyroiditis and chronic periodontitis (5,34,35,41–43).

In *in vitro* investigations on material obtained from patients with peptic ulcers of the stomach and the duodenum, rheumatic fever, autoimmune thyroiditis, polymyositis and pyelonephritis, increased adhesion of lymphocytes to the HECs was described (34).

HECs were also shown to play similar functions as monocytes and macrophages. They produce cytokines such as interleukin (IL)-1 and colony-stimulating factor (42,44,45).

HEVs also play an important role in scavenging injured and apoptotic lymphocytes (46,47). Expression of Fas receptor ligand was demonstrated on HECs in reactive human lymph nodes, confirming involvement of the cells in the induction of Fas/Fas ligand-dependent apoptosis (48).

HEVs and periodontal diseases

In biopsies of inflamed gingival tissues there was evidence for both angiogenesis and deposition of perivascular hyaline material (basal lamina components), which can impair the emigration of polymorphonuclear leukocytes (PMNs) into the gingival sulcus (49).

In inflamed gingiva and advanced chronic periodontitis, several authors observed periodontal HEV-type blood vessels (PHELVs) (50,51). In periodontitis the connective tissues manifest an inflammatory infiltrate consisting mainly of T and B lymphocytes (52–54), frequently surrounding HEVs (Fig. 1B). These specialized vessels were not detected in healthy gingiva (15,51). Proliferation of blood vessels under the junctional epithelium has also been described in periodontal diseases in chimpanzees: this involved capillary loops containing high numbers of neutrophils and extending to approach the attenuated pocket epithelium (46). Many intact PMNs were observed intracellularly within the connective tissue and the epithelium also by other authors. Several layers of PMNs surrounded the plaque mass (55). Pinchback *et al*. demonstrated that substances released from dental plaque have easy access to connective tissue and the vascular system. Accumulation of lymphoid cells was accompanied by atrophy of collagen fibers, hyperplasia of loose connective tissue and proliferation of capillaries (56).

In patients with chronic periodontitis a disturbed immune response can be noted, as a result of altered functions of neutrophils, monocytes, lymphocytes and fibroblasts (57). Vascular expansion encompassing augmented luminal diameters, increased tortuosity and appearance of HEVs is distinctive. Changes in vascular structure, the involvement of vasculature overproduction of cytokines and phagocytic properties of HECs indicate that the vasculature contributes to the progressive lesion (41,58,59). These characteristics support the inclusion of chronic periodontitis in the category of vasoproliferative diseases (60).

The appearance of HEVs in tissues of the oral cavity may reflect the action of several factors, including the up-regulation of vascular endothelial growth factor which exerts pro-angiogenic activity and augments the permeability of blood vessels. Vascular endothelial growth factor is considered to be responsible for bleeding and swelling of gingiva (61). ECs produce cytokines and adhesion factors for lymphocytes and for other inflammation-promoting substances, thus assisting in the appearance of new lymphocyte populations in the inflammatory focus. Monocytes and macrophages also take part in the process of angiogenesis because they are able to produce factors responsible for the process (44,61). The production by endothelium of growth factors, including vascular endothelial growth factor, basic fibroblast growth factor, cytokines and chemoattractants, amplifies angiogenesis and increases the migration of proinflammatory cells (41,42,51).

Expression of selected surface markers of HEV necessary for leukocyte transendothelial migration

Work in the authors' laboratory focused on the characterization of HEC-linked adhesive molecules indispensable for each stage of leukocyte migration into lymphoid/nonlymphoid tissues (e.g. gingiva in chronic periodontitis) (described in Fig. 3).

The adhesive cascade is known to include, on the one hand, adhesive molecules belonging to selectin, integrin and sialomucin families and, on the other hand, molecules belonging to the superfamily of immunoglobulins, expressed by both lymphocytes and HEVs (6,8,16,25).

CD34

The CD34 molecule is a single-chain, transmembrane glycoprotein. Together with podocalyxin and endoglycan, CD34 is grouped in the CD34 family and the subfamily of sialomucins (62). Molecules of this family are specifically expressed by HEVs and react with L-selectin present on most leukocytes (8,63) (Fig. 3). When related to HEV function, CD34 family proteins are classified as members of the peripheral node addressins family (8).

In humans, CD34 has a molecular mass of 105–120 kDa (64–66), while the molecular mass of the murine form of CD34 ranges from ~90 to ~100 kDa (67). The gene encoding human CD34 contains eight exons and is located on chromosome 1 (64,68).

Pioneering studies on CD34 were directed towards analysis of expression as a marker to assist in the identification and isolation of HSCs and progenitors in preparation for bone marrow transplantation (62). CD34 was present as a 110-kDa product on hematopoietic progenitor cells/hematopoietic stem cells and also on the vascular endothelium (69). CD34 is expressed on small blood vessels in the majority of tissues in normal organs and on several stem cell and neoplastic cell lines (67,69). *CD34* mRNA was demonstrated in cultured umbilical vein endothelial cells (HUVECs) as well as in other tissues rich in vascular endothelium (breast and placenta) (69) and in freshly cultured vascular ECs (70). CD34 is not expressed in the endothelia of large blood vessels or placental sinusoids (69). Expression of CD34 on ECs was shown to be maintained at a high level on the luminal surface while basolateral aspects

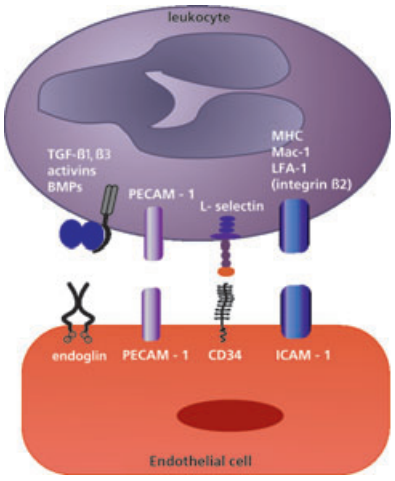

Fig. 3. The adhesion molecules [endoglin, platelet endothelial cell adhesion molecule 1 (PECAM-1), CD34 and intercellular adhesion molecule 1 (ICAM-1)] expressed by high endothelial cells (HECs) and their counterpart receptors on leukocytes [lymphocytes, monocytes and polymorphonuclear leukocytes (PMNs)] involved in the multiple adhesion cascades are shown. The leukocytes initiate rolling via interactions between L-selectin and peripheral node addressins (including CD34). Subsequently, leukocytes adhere firmly to the luminal surface of high endothelial venules (HEVs), mainly via interactions between leukocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule (ICAM)-1 and -2. PECAM-1 mediates PECAM-1 homophilic adhesion and participates in firm adhesion and transmigration. Endoglin probably takes part in lymphocyte adhesion to the luminal surface of HEVs, but its precise role in transendothelial migration remains unclear.

showed weaker expression (66,71). Interestingly, in hematopoietic progenitor cells, CD34 manifests in both membranous and cytoplasmic forms. Cultured HUVECs demonstrate a diffuse membranous expression of CD34 (partially also on the surface of microvilli) (70). Endothelial cell expression of CD34 is thought to accompany development of blood vessels at every stage, in physiological conditions and on recapitulation of angiogenesis in neoplastic lesions (72).

The characteristics of CD34 are shown in Table 1.

CD34-positive cells were found to include a large number of different cell types (62,70,73–75) (Tables 1 and 2). In addition, overlapping expression was detected for CD34 and the CD34-related proteins, podocalyxin and endoglycan (reviewed in 62).

In HEVs in peripheral lymphoid tissues (lymph nodes and tonsils), CD34 was expressed also on the free surface of HECs (63,76). Subcellular localization of CD34 is summarized in Table 3.

The exact function of CD34 remains unknown but several relevant hypotheses have been presented. A decrease in expression of CD34 protein and CD34 mRNA was noted following administration of IL-1β, interferon-γ and tumor necrosis factor-α (TNF-α), under conditions in which the ligands upregulated the expression of endothelial leukocyte adhesion molecule 1 (selectin E) and intercellular adhesion molecule 1 (ICAM-1) (70). Recent *in vitro* studies have confirmed that expression of ICAM-1, vascular cell adhesion molecule 1 (VCAM-1), MAdCAM-1 and selectin E increases in mouse lymphatic endothelium as a result of stimulation with the proinflammatory cytokines IL-1β, TNF-α and interferon-γ. In human ECs of lymphatic vessels the cytokines induced increased expression of EC adhesion molecule, but not of MAdCAM-1 (90). CD34 proved to be a ligand for L-selectin (CD62L), which is expressed constitutively on the cell membrane of leukocytes (Fig. 3). The lectin domain of L-selectin recognizes carbohydrate residues (sulfated, fucosylated and sialated types) of the CD34 extracellular fragment (76). Using MECA-79 monoclonal antibody, intense cytoplasmic staining as well as luminal and abluminal reactivities were demonstrated for HEVs (91).

Involvement of CD34 in leukocyte transendothelial migration and inflammation— The best-documented function of CD34 involves its engagement in the promotion of lymphocyte adhesion to HEVs in lymphatic organs and, more precisely, at stages 1 and 2 of leukocyte passage through HEVs (rolling and activation of lymphocytes) (71,76). CD34 was found to bind L-selectin, representing the first step of the movement of naive lymphocytes into secondary lymphoid organs (63) (Fig. 2).

CD34 plays an important role at various stages of inflammation, and its expression is maintained at the sites of a developing inflammatory process. The role of CD34 on the surface of other cells involves stimulation of cell motility and more effective migration into the affected tissues (73,74). Involvement of CD34 antigen is also speculated to affect the dynamic properties of mature human fibroblasts (75).

Platelet endothelial cell adhesion molecule 1

Platelet endothelial cell adhesion molecule 1 (PECAM1) (EndoCAM) is a glycoprotein of 130 kDa molecular mass, and a member of type I transmembrane adhesion molecules and the superfamily of immunoglobulins (92,93). The primary cellular target for human PECAM-1 is PECAM-1, and the immunoglobulin homology domains 1 and 2 mediate PECAM-1 homophilic adhesion (94) (Fig. 2).

PECAM-1 consists of a cytoplasmic domain (in the form of a long tail), a short hydrophobic transmembrane domain and six extracellular domains, resembling in structure immunoglobulin chains (95). At least two of the domains manifest an adhesive potential (96,97). They are responsible for contact with other molecules, and are mediators of adhesion, participating in establishing contact between ECs and stabilizing the endothelial structure (96–98). The cytoplasmic domains contain two special immunoreceptor tyrosine-based inhibitory motifs responsible for transfer of an inhibitory signal in a cell, as well as a motif with an activating sequence, the immunoreceptor tyrosine-based activation motif (99,100).

Cellular expression of PECAM-1 is manifested on different lineages of cells (101–104) (Table 2). In ECs, the expression of PECAM-1 is particularly high in lateral cell membranes, at the site of intercellular junctions. In such sites their concentration reaches 10^6 molecules, fulfilling a binding role in inflammatory and immune processes as

Table 1. Characteristics of selected adhesion molecules necessary for leukocyte transendothelial migration in postcapillary high endothelial venules

	CD34	PECAM-1	Endoglin (CD105)	ICAM-1 (CD54)
Gene/chromosome localization	1q32	17q23	9q33-34	19p13.3-p13.2
Transcript size	26 kb	1kb		3.3 kb
Protein size	105–120 kDa (385 amino acids)	130–140 kDa (738 amino acids)	180 kDa (633 amino acids)	70–120 kDa (532 amino acids)
Cell types with major expression	Human hematopoietic progenitor/stem cells, endothelial cells of small blood vessels (including high endothelial cells)	Venous endothelial cells (including high endothelial cells), platelets	Endothelial cells (including high endothelial cells)	Endothelial cells (including high endothelial cells)
Other cells with expression	Multipotent precursor cells, mouse mast cells, human fibroblasts and fibrocytes, hematopoietic cancer cells	Leukocytes (granulocytes, monocytes, neutrophils), smooth muscle cells, unique T-cell subset and B-cell subpopulation, cancer cells	Syncytiotrophoblasts, stromal cells, certain hematopoietic cells, muscle cells, fibroblasts, activated macrophages, cancer cells	Leukocytes, epithelial cells, fibroblasts, cancer cells
Role in adhesive cascade	First and second steps of transendothelial migration (rolling and leukocyte activation)	Third and fourth steps of transendothelial migration (leukocyte adhesion and diapedesis)	Third step of transendothelial migration (leukocyte adhesion to high endothelial cells)? Fourth step of transendothelial migration?	Third and fourth steps of transendothelial migration (leukocyte adhesion and diapedesis)
Other functions	Inhibition of mast cell adhesion and aiding appropriate homing of mast-lineage cells in mice	Angiogenesis, integrin activation, thrombocyte aggregation	Angiogenesis, maintaining the homeostasis of vessels, endothelial cell differentiation, vascular repair, bone marrow mesenchymal stem cell regulation	T-cell activation (immunological synapse), presenting the antigen to cytotoxic T cells, receptor for human rhinoviruses, induction of many proinflammatory paths and regulation of cytokines
References	Baumhueter *et al.* (63), Simmons *et al.* (64), Benedetti (65), Kikuta & Rosen (66), Krause *et al.* (67), Satterthwaite *et al.* (68), Fina *et al.* (69), Drew *et al.* (73), Drew *et al.* (74), Barth & Westhoff (75), Rosen (76)	Gumina *et al.* (92), Newman (93), Xie & Muller (95), DeLisser *et al.* (98), Sheibani & Frazier (101), Tang *et al.* (103), Tanaka *et al.* (104), Muller *et al.* (107), Cao *et al.* (111), Tachezy *et al.* (112)	Chaifetz *et al.* (116), Fernàndez-Ruiz *et al.* (118), Kumar *et al.* (124), Bodey *et al.* (125), Fonsatti *et al.* (126), Torsney *et al.* (129), Burrows *et al.* (130), Dallas *et al.* (131), van Laake *et al.* (132), Bühring *et al.* (131), Conley *et al.* (142), Meunzner *et al.* (145)	van de Stolpe & van der Saag (148), Bossy *et al.* (149), Cook-Mills & Deem (152), Adamson *et al.* (160), Wojciak-Stothard *et al.* (161), Stinchcombe *et al.* (166), Lebedeva *et al.* (169), Blaber *et al.* (170), Shaw *et al.* (177), Millan *et al.* (180)

ICAM-1, intercellular adhesion molecule-1; PECAM-1, platelet endothelial cell adhesion molecule 1.

well as in processes associated with thrombocyte aggregation (96). In the case of HEV endothelium, expression of PECAM-1 is localized to both luminal and lateral aspects of the cell membrane (8) (Table 3).

From the clinical point of view, the link between PECAM-1 and the function of endothelium in veins seems to be particularly important (105,106).

Data on the molecular structure and the role of PECAM-1 in inflammatory and immune processes are summarized in Table 1.

Involvement of PECAM-1 in leukocyte transendothelial migration and inflammation— In the relatively rich literature describing the function of PECAM-1, participation in transendothelial migration was stressed (94,95,106–110). PECAM-1 takes part in the third and fourth steps of transendothelial migration, as demonstrated by functional studies (107). The first reports on adhesive properties of the molecule originate in 1991, from studies by Albelda *et al.* (106), and the data on involvement of PECAM-1 in transendothelial migration of leukocytes were supplied in 1993 by Muller *et al.* (107) and in 1994 by Bogen *et al.* (108). In the same year these observations were confirmed by Vaporciyan *et al.* (109) in studies on traffic of neutrophils in an animal model. An involvement of PECAM-1 was suggested in the passage of monocytes through the endothelial basement membrane as a result of interactions of the molecule with components of the extracellular matrix. On the other hand, the molecule seems to play a less important role

Table 2. Cell-type distribution of surface adhesion molecules in human and animal tissues

Type of cell	CD34	PECAM-1	Endoglin	ICAM-1
Endothelial cells	+	+	+	+
High endothelial cells	+	+	+	+
Multipotent precursors	+	–	+	–
Hematopoietic precursors/stem cells	+	–	–	–
Progenitor and adult mast cells	+			
Bone marrow stromal cells			+	
Megakariocytes/platelets	–	+	–	–
Monocytes/macrophages/osteoclasts	–	+	+	+
Dendritic cells				+
Neutrophils		+		
Eosinophils	+			
Erythrocytes	–	–	–	–
Fibroblasts/fibrocytes	+	–	+	+
Plasma cells				+
Subsets of T and B cells		+		+
Epithelial cells	–	–		+
Smooth muscle cells		+	+	
Cardiac muscle cells	+			
Adult and/or fetal fibroblasts	+	–	+	+
Tumor cells and/or tumor cell lines	+	+	+	+

ICAM-1, intercellular adhesion molecule-1; PECAM-1, platelet endothelial cell adhesion molecule 1.
CD34: Nielsen & McNagny (62), Krause *et al.* (67), Satterthwaite *et al.* (68), Fina *et al.* (69), Delia *et al.* (70), Drew *et al.* (73), Drew *et al.* (74), Barth & Westhoff (75).
PECAM-1: Tohya *et al.* (8), Sun *et al.* (94), Sheibani & Frazier (101), Golberger *et al.* (102), Tang *et al.* (103), Tanaka *et al.* (104), Muller *et al.* (107).
Endoglin (CD105): Izawa *et al.* (12), Balza *et al.* (77), Jen *et al.* (78), Ozbey *et al.* (79), Kestendjiewa *et al.* (80), Gougos & Letarte (117), Barnabeu *et al.* (121), Kumar *et al.* (124), Bodey *et al.* (125), Fonsatti *et al.* (126), Burrows *et al.* (130), Dallas *et al.* (131), Postiglione *et al.* (133), Gougos & Letarte (134), Bühring *et al.* (136), Robledo *et al.* (137), Cook-Mills *et al.* (146).
ICAM-1 (CD54): Perry *et al.* (81), Cook-Mills & Deem (152), Simmons *et al.* (153), Marlin & Springer (154), Dustin & Springer (175), Sasaki *et al.* (179), Millan *et al.* (180).

in a similar passage of lymphocytes as inactivation of the PECAM-1-coding gene failed to disturb the passage of leukocytes in lymph nodes (110). Another role of PECAM-1, stressed by reports, is its involvement in angiogenesis (111,112).

The molecular mechanisms that result in the synthesis of PECAM-1 by cells continue to pose a dilemma. The adhesive properties of the extracellular domains may be regulated by the cytoplasmic domain. HECs devoid of PECAM-1 formed no tight junctions and did not manifest a typical cubic structure (98). Modifications in the cytoplasmic domain of the molecule affect its adhesive properties and the binding of appropriate ligands (105,113). PECAM-1 participates in apoptosis and signal transduction (99), including signals that inhibit programmed cell death (100). The mechanism of the cytoprotective activity of PECAM-1 remains unknown; it probably involves binding to various enzymes, such as kinases and phosphatases. Involvement of the protein is assumed in the control of transcription of apoptotic proteins (99). It was demonstrated that tumor cell PECAM-1 is involved in mediating tumor cell adhesion to endothelium (103). In turn, studies of the mechanisms through which the molecule acts in the transport of leukocytes across ECs provide evidence for its involvement in the control of the signaling pathway with involvement of Ca^{2+} ions. Activation of calcium channels is believed to play a role in the transmigration of neutrophils (114). Despite the broadly implemented studies, the function of PECAM-1 has not been fully clarified. Further investigations on the molecule may result in a therapy that eliminates unwanted inflammatory reactions and other pathological conditions (115).

Endoglin (CD105)

Endoglin represents a homodimeric integral membrane type 1 glycoprotein, composed of two disulfide-linked subunits of 90–95 kDa (116). It is described as a marker protein of active, proliferating ECs in capillaries, arterioles and veins. Expression of endoglin was demonstrated on the membrane of ECs in all tissues except for bone marrow (117).

Endoglin consists of a 561-amino-acid extracellular domain, a 25-amino-acid hydrophobic transmembrane domain and a 47-amino-acid intracellular tail, representing the cytoplasmic domain (117). The endoglin-coding gene is situated on chromosome 9 (9q33-34) (118).

The presence of an Arg-Gly-Asp (RGD) motif, located in the zona pellucida domain of the extracellular region of endoglin, suggests its role in the process of reciprocal binding between ECs and integrins on the one hand and other receptors for the RGD on the other. The RGD motif is responsible for adhesion and cell aggregation. It also participates in cell interactions with the extracellular matrix (117). The 47-residue cytosolic domain of the predominant L-isoform of CD105 constitutes a region of the protein with the highest degree of conservation among endoglins from different mammalian species, as well as with the homologous protein, betaglycan (119).

Data on the molecular structure and on the main role of endoglin in the context of leukocyte migration are summarized in Table 1.

In a functional respect, endoglin represents an accessory protein of the multiple kinase receptor of the transforming growth factor β (TGF-β) superfamily and modulates TFG-β-dependent cellular responses (116,120,121) (Fig. 3). Overexpressed endoglin is able to modulate cellular responses to TGF-β in several cell types, including ECs (122,123). CD105 is markedly up-regulated in the proliferating endothelium of tissues undergoing angiogenesis (124–126). CD105 binds TGF-β1 and

Table 3. Cellular and subcellular localization of surface adhesion molecules in high endothelial cells

Adhesion molecule	Cellular and subcellular localization
CD34	Luminal and abluminal surfaces of high endothelial cells Microvillous processes near the endothelial cell junctions Golgi apparatus and some vesicular structures of high endothelial venules Subset of electron-dense granular structures
PECAM-1	Luminal surfaces of high endothelial cells Apical surface of the endothelial cells over the intercellular junction Lateral membrane of high endothelial cells Microvillous processes of high endothelial cells at the lateral membrane Intraendothelial contacts and vesicle-like structures along the cell border
Endoglin (CD105)	Luminal surfaces and cytoplasm of high endothelial cells
ICAM-1 (CD54)	Luminal and lateral surfaces of high endothelial cells Over the luminal surfaces, which undulated to form microfolds and shallow microfurrows Icrovillous processes of high endothelial cells

ICAM-1, intercellular adhesion molecule-1; PECAM-1, platelet endothelial cell adhesion molecule 1.
CD34: Tohya *et al*. (8), Kikuta & Rosen (66), Baumhueter *et al*. (71), Streeter *et al*. (78) & Girard *et al*. (82).
PECAM-1: Tohya *et al*. (8), Muller *et al*. (107), Pfeiffer *et al*. (83), Ayalon *et al*. (84), Jin *et al*. (85), Mamdouth *et al*. (86,87).
Endoglin (CD105): Kasprzak *et al*. (229), Tomczak (230).
ICAM-1 (CD54): Tohya *et al*. (8), Perry *et al*. (81), Brown *et al*. (88), Tanaka *et al*. (89), Sasaki *et al*. (179).

TGF-β3 by associating with the TGF-β type II receptor and interacts with activin-A and bone morphogenetic protein-7 (BMP-7) via activin type II receptors, ActRII and ActRIIB, regardless of which type I receptor partner is co-expressed (120). Endoglin has also been shown to interact with the TGF-β type I receptor (120,127,128).

In physiological conditions, expression of CD105 is insignificant, whereas in pathology it is detected in proliferating ECs within inflammatory sites, in tumors and in regenerating tissues (129–132). CD105 is considered to provide an important marker of neoangiogenesis in neoplastic cells (112,133). Endoglin was originally identified in human vascular ECs and was shown to be highly expressed on HUVECs in culture (134). Other cells producing endoglin (121,134–137) are cited in Tables 1 and 2.

Endoglin plays a role in bone marrow mesenchymal stem cell regulation (138), in formation of haemangioblasts (139) and in blood mononuclear cell-mediated vascular repair (132). Mutations of the endoglin gene were demonstrated in certain diseases linked to disturbed vasculogenesis (e.g. in hereditary hemorrhagic telangiectasia) (140). Lack of CD105 expression was documented also in diseases linked to lung developmental disturbances or in fetuses with cardiac malformations (132,141).

The functions of endoglin are most frequently associated with angiogenesis, adhesion and cell migration, as well as with maintenance of vascular homeostasis (reviewed in 121). Endoglin affects the differentiation of the EC phenotype in many tissues, including HEV-type blood vessels (12,132). The most important functional fragment of the molecule involves the cytoplasmic domain (121). Endoglin expression results in the inhibition of cell migration in *in vitro* and *in vivo* models (121). The process is controlled by endoglin–zyxin interactions (142) and with the cytosolic protein, zyxin-related protein 1 (143). Interactions with these proteins result in the redistribution of zyxin-related protein 1 from sites of focal contacts to F-actin stress fibers in ECs and in a dynamic rearrangement of F-actin fibers (143). The interactions between endoglin and betaglycan are also significant (121). Endoglin interacts with protein Tctex2b (from the Tctex1/2 family of cytosolic dynein light chains), linking endoglin to microtubule-based transport (144). The cytosolic domain of endoglin also plays a role in amplifying cellular adhesion with involvement of integrin β1 (145).

Positive expression of endoglin, in line with the other HEC markers, was demonstrated in cultured ECs established from BALB/c mouse axillary and cervical lymph node and in HECs of lymph node tissue sections (146). Actively expressed genes in HECs of mouse lymph node, including the gene encoding endoglin, were catalogued by Izawa *et al*. (12). Expression of endoglin in HECs was more pronounced than expression of PECAM-1 or CD34 and it was detected with a markedly higher frequency than on PECAM-1-positive cells of the flat vascular endothelium (12).

Involvement of endoglin in leukocyte transendothelial migration and inflammation The precise mechanism of action of endoglin in the leukocyte adhesive cascade has not been described (8,20). As noted, the literature implicates endoglin in the regulation of integrin-mediated cell adhesion and detachment (145). The molecule may directly mediate important cell-adhesive, proliferative and migration processes in the developing and adult vasculature (121) (Table 1).

Intercellular adhesion molecule-1

ICAM-1 (CD54) is an inducible surface glycoprotein of 70–120 kDa molecular mass (147,148). The primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families (134). The gene encoding human ICAM-1 is positioned on chromosome 19 (149,150) (Table 1). The mature molecule of ICAM-1 consists of five immunoglobulin-like extracellular domains (D1–D5) (of 453 amino acids), a short transmembrane region and a

small carboxyterminal cytoplasmic domain (of 28 amino acids), the final fragment of which links the molecule to the cytoskeleton through α-actinin and β-tubulin (147,148,151). The extracellular domains of ICAM-1 are responsible for binding ligands and contain potential sites for glycosylation (152). The second, third and fourth immunoglobulin domains are heavily N-glycosylated, with four potential sites in D2, two in D3 and two in D4 (151,153).

Under normal conditions, ICAM-1 acts as an endothelial receptor for leukocyte function-associated antigen-1 (LFA-1, CD11a/CD18, integrin β2), an αβ complex that is a member of the integrin family of cell–cell and cell–matrix receptors and is expressed on leukocytes (147,154,155) (Fig. 3). The ICAM-1 and LFA-1 interaction is heterophilic, and adhesion is primarily between the D1 domain (155) and the insertion domain (156). Unlike other integrin ligands, ICAM-1 does not contain an RGD motif, but has a larger, more extended, binding surface (153,155,157). Upon ligation, ICAM-1 activates RhoA family G-proteins, small guanosine triphosphatases (GTPasaes) (158,159) and induces the polymerization of actin, preconditioning strict adhesion and passage of leukocytes through the vascular endothelium (160,161). ICAM-1 occupancy triggers elevation in intracellular free Ca^{2+} and myosin contractility (162), and activation of p38 kinase (163) and the tyrosine kinase $p60^{Src}$ (164). Activation of these signaling pathways induces extensive cytoskeletal remodeling events that alter EC contractility and function, possibly facilitating leukocyte diapedesis (163).

ICAM-1 on the surface of HECs binds also to (apart from LFA-1) macrophage adhesion ligand-1 (Mac-1, ITGB2, ITGAM), which is expressed on leukocytes (8,157) (Fig. 3). Recent data indicate that ICAM-1 and major histocompatibility complex molecules might also contribute to molecular segregation at the so-called immunological synapse, thereby facilitating T-cell activation (165,166).

ICAM-1 is subverted as a receptor by the major group of common cold human rhinoviruses. Analyses showed that rhinoviruses mimic LFA-1 in binding to the most membrane-distal site of ICAM-1 (155). This was confirmed by subsequent studies, demonstrating that rhinovirus attachment is confined to the BC, CD, DE and FG loops of the amino-terminal immunoglobulin-like domain (D1) at the end distal to the cellular membrane (157). Apart from LFA-1 and human rhinoviruses, ICAM-1-binding sites for fibrinogen (148) and malaria-infected erythrocytes were also demonstrated (167,168).

The role of ICAM-1 in immune and inflammatory processes is more complex than was thought in earlier studies. Apart from the well-documented role of ICAM-1 as an adhesion molecule, the role of the molecule is stressed as a costimulator, facilitating antigen presentation to cytotoxic T cells during major histocompatibility complex-I-restricted antigen presentation (reviewed in 169). ICAM-1 induces many signaling pathways (mainly pro-inflammatory) in the cell, including Abl tyrosine kinase, SCR-family kinases and p38 MAPK, suggesting a role for ICAM-1 in mediating inflammation. The physiological role of such activation (particularly activation of kinases) involves increased production of cytokines and cell-surface proteins such as major histocompatibility complex-I, IL-1 receptor, VCAM-1 and regulated on activation, normal, T-cell expressed, and secreted (RANTES) (169,170). ICAM-1 ligation was found to up-regulate ICAM-1 expression in a positive-feedback loop. ICAM-1 might also function as cell-surface receptor, capable of initiating intercellular signaling, which is implicated in the regulation of cytokine production (IL-1β, IL-6, IL-8 and IL-12) during the course of cell proliferation and differentiation as well as in the regulation of some membrane proteins, including immune receptors (reviewed in 169).

A circulating (soluble) form of ICAM-1 (cICAM-1 or sICAM-1) was also identified for the first time in normal human serum and in sera from patients with leukocyte adhesion deficiency (171). This form of ICAM-1 can also be detected in cerebrospinal fluid and in gingival fluid (148,172,173). In healthy individuals, the serum levels of cICAM-1 range from 100 to 200 ng/mL, while markedly elevated levels (200–700 ng/mL) are detected in patients with leukocyte adhesion deficiency (171). Serum levels of cICAM-1 may provide indirect proof for an inflammatory condition or tissue injury.

ICAM-1 is expressed constitutively on the surface of several cell types, including leukocytes, fibroblasts, epithelial cells and ECs (153) (Table 2). Endothelium expresses low levels of ICAM-1 and inflammatory stimuli can markedly increase the surface expression of ICAM-1 (152,174,175).

Involvement of ICAM-1 in leukocyte transendothelial migration and inflammation ICAM-1 protein that promotes adhesion in immunological and inflammatory reactions. The normal function of the molecule is to provide adhesion between ECs and leukocytes after injury or stress (step 3 of transendothelial migration) and to participate in diapedesis (step 4 of transendothelial migration) (23,147,148) (Fig. 2). Involvement of ICAM-1 in leukocyte transendothelial migration is linked to the activation of cell signals that control mainly cytoskeletal functions in HECs (148,152). Of note, the studies on the role of endothelial expression of ICAM-1 in transendothelial migration, in the model of TNF-α-stimulated HUVECs, showed that all three types of leukocytes (granulocytes, monocytes and lymphocytes) migrate through the wall of such blood vessels. In these studies it was demonstrated that 5–10% of leukocytes pass directly through the cytoplasm in individual venules (transcellular transport), most traversed between ECs (paracellular transport) while the remaining leukocytes showed no clearly outlined path of migration. Interesting were the observations showing that the paracellular and transcellular diapedesis was correlated with the presence of cup-like structures, rich in adhesive molecules (ICAM-1 and VCAM-1), arranged in parallel to the leukocyte migration path (176,177). A model was also presented for *in vitro* transcellular passage of PMNs, in which

PMNs, but not $CD3^+$ T lymphocytes, were shown to undergo transcellular migration via a mechanism involving high-occupacy levels of ICAM-1 on the endothelial surface (178).

Studies on the three-dimensional location of ICAM-1 on the surface of HECs showed that the molecule undergoes a most pronounced exposure on the luminal surface of HEV, forming microfolds and grooves (179) (Table 3). Translocation of ICAM-1 to caveolin-rich membrane domains close to the ends of F-actin stress fibers controls transcellular diapedesis of human T lymphocytes (180). On the lymphocytes that were apposed to HECs, LFA-1 was shown to form capping and clustering patterns, indicating that the topology of LFA-1 is also dynamically regulated during the course of lymphocyte–HEV interactions (181). The functional and morphological studies of Shulman *et al.* (182) demonstrated that T cells crawl in a millipede-like manner on ICAM-1, expressing ECs using their LFA-1-dependent filipodia. Other authors negate this suggestion, observing, in scanning electron micrographs, that the great majority of lymphocytes in the adhesion step or the early step of transendothelial migration in HEVs retain a relatively spherical cell body rather than an elongated shape that would be required for millipede-like movement (8).

Endothelial expression of adhesion molecules in periodontal diseases

The most important etiological factor of chronic periodontitis involves bacteria and probably also viruses (183,184). The variable immune response of the host to pathogens, and the progress and intensity of inflammatory lesions in chronic periodontitis, depends on genetic and environmental factors (183–185). Inflammatory processes, in conjunction with immune responses to bacteria, are generally protective. In profound chronic periodontitis, hyper-responsiveness and hypersensitivity of the immune system leads to a destructive process, affecting the supportive structures of the teeth, resorption of alveolar bone and formation of periodontal pockets (186,187). In the course of the disease, the gingival tissue, well supplied with blood vessels, responds to infectious stimuli. The augmented blood vessel proliferation (51,59,60) and an increased number of circulating endothelial progenitor cells (EPCs) have a clear rationale (188). It has been proven that the number of EPCs increases upon development of moderate to severe chronic periodontitis, compared with individuals with no or only mild chronic periodontitis. In such patients the higher number of EPCs and EPCs of a more mature phenotype ($CD34^+/KDR^+$) is positively correlated with the concentrations of C-reactive protein (188). Recent observations seem to confirm that periodontitis is an independent risk factor for systemic vascular disease and may reflect stimulation of acute-phase protein synthesis by cytokines released by periodontal HECs (50,188–192). Expansion of microvessels in chronic periodontitis through increased vascular diameter and tortuosity, as well as the development of HECs, appears to protect from periodontitis by increasing the supply of both plasma defense factors and PMNs to the tissues (15,50).

Pioneering studies involving demonstration of PHELVs in the gingiva of patients with chronic periodontitis were published in the 1990s (51). In the lumen, the blood vessels contained mainly PMNs (51). Similarities of the blood vessels were demonstrated to typical HEVs of lymph nodes, also at the ultrastructural level. Histochemical techniques defined a particularly strong activity of acid phosphatase in PHELVs and the presence of 5′-nucleotidase in 50% of PHELVs of the gingiva. Moreover, similarly to lymph-node HEVs, PHELVs selectively incorporated $^{35}SO_4$ (51). It was concluded that gingival HEVs may represent sites for migration and recirculation of leukocytes in the inflamed periodontal tissues as no such blood vessels were seen in healthy gingiva (15,51). For the first time, the prevalence of PMNs over lymphocytes (even in the absence of lymphocytes) in transendothelial migration was demonstrated in chronic periodontitis (51). Only in earlier gingivitis lesions are more T lymphocytes (positive for CD11a, CD25, and CD4) found to migrate into tissues (193). According to pioneers of studies on gingival HEVs in chronic periodontitis, the maintenance of the high endothelium phenotype in the blood vessels may reflect not, as assumed previously, a prolonged and extensive lymphocyte emigration, but locally produced factors (51).

It is generally accepted that PMNs play a protective role in periodontal lesions (55). However, the location, incidence and extent of perivascular hyaline material promotes periodontitis by inhibiting the migration of PMNs (42,49). Studies have shown that the perivascular material in chronic periodontitis most frequently involves collagen type IV and laminin, components of basement membranes in mainly venous blood vessels but deposited also extravascularly (49,56).

In chronic periodontitis the quantitative vascular lesions were shown to be accompanied by qualitative alterations, including dilation of basement membrane in capillaries and venules (41,49,56,58). In the disease, a quantitatively variable expression of adhesive molecules is noted on ECs (including HECs), which is linked to the role of ECs in the process of transendothelial migration (194,195). The adhesive molecules were suggested to represent one of the main elements which define the course of the disease (185).

From the references reviewed in this paper, the results of studies were selected on the basis of at least an indirect relationship to transendothelial migration across HEVs present in the inflamed tissues of the periodontium. The most numerous studies on the expression of adhesion molecules in the development and progression of periodontal diseases pertain to ICAM-1 (CD54) (172,185,194,196–200). This molecule was even termed an 'inflammation biomarker' for periodontitis (201). Analysis of the cICAM-1 form in gingival fluid demonstrated an increase in the release of protein to the intercellular fluid, which correlated with the build-up of dental plaque and

with intensity of the inflammatory condition (173). Studies on the serum concentration of soluble adhesion molecules, including cICAM-1, in patients with chronic and aggressive periodontitis, demonstrated a significant increase of the protein in both forms of periodontitis compared with the control group (199).

ICAM-1 was detected in various types of cells (epithelial cells, ECs and leukocytes) of gingiva, suggesting roles in the consecutive stages of periodontitis (193,196,202). Expression of the molecule was described in the capillary loops of gingiva affected by inflammatory condition, but, in contrast to PECAM-1, expression was detected in a proportion of the blood vessels only (203). Both adhesion molecules – ICAM-1 and PECAM-1 – were detected on ECs and keratinocytes of gingival epithelium affected by gingivitis and periodontitis and the degree of expression was dependent on the size of the inflammatory infiltrates (197). An interesting study was devoted to the effect of tobacco smoking (which affects the systemic concentration of cICAM-1) on the local expression of the marker in patients with chronic periodontitis (204). Investigations documented an increased expression of ICAM-1 in sites with more pronounced inflammatory lesions, manifesting no relationship to tobacco smoking, but the proportion of the total number of vessels expressing ICAM-1 in noninflamed sites was greater in nonsmokers than in smokers. The authors confirmed the observations that tobacco smoking exerts a systemic effect on ICAM-1, independent of local inflammation (204). Other studies on the cellular expression of ICAM-1, linked to analysis of vascular density in gingiva and to expression of β1 integrin, demonstrated no significant differences in chronic periodontitis patients who responded to treatment and patients who failed to respond to initial treatment (185).

The specific cells in gingival inflammatory infiltrates that expressed ICAM-1 were identified. A proportion of investigators observed expression mainly on T lymphocytes (50% involved LFA-1^+ and $CD29^+$ T lymphocytes) and documented similar values of lymphocytic expression of ICAM-1 in the gingiva of patients with gingivitis and those with periodontitis (197,205).

ICAM-1 was also localized in keratinocytes of the epithelium of the periodontal pocket (193,197,202). It should be noted that such a location was always common to expression of the molecule on ECs of gingival blood vessels and leukocytes in inflammatory infiltrates. Topographically, ICAM-1 was demonstrated both in the normal epithelium lining healthy gingivae and in the pocket epithelium in diseased gingiva (196,202). As a rule, the location involved deeper layers of epithelial keratinocytes (196,202). Increased expression of ICAM-1 and LFA-1 was detected in junctional epithelium and in the apical part of the sulcus epithelium in subjects with chronic periodontitis compared with controls (202). The expression on keratinocytes increased in parallel to the size of the inflammatory foci (197). Interestingly, cells positive for *IL8* mRNA were also detected, and a gradient of ICAM-1 receptors within the junctional epithelium of clinically healthy gingiva, the area of PMN migration, was established (198). The authors suggested that ICAM-1 and IL-8 play important physiological roles in efficiently routing PMNs to the gingival sulcus (198). In turn, Takeuchi *et al*. (193) showed that expression of ICAM-1 in pocket epithelium in periodontitis is relevant to the migration of the infiltrating lymphocyte population (CD11a, CD25 and CD4 positive) in connective tissue subjacent to the pocket epithelium in the periodontal pocket. Functional studies *in vitro* on involvement of the water-channel protein, aquaporin 3 in chronic periodontitis, documented increased expression of this protein and of ICAM-1 in keratinocytes of the gingival epithelium. The studies discovered that a reduction in aquaporin 3 expression of more than 65% significantly attenuated selected proinflammatory events of ICAM-1 expression that were induced by TNF-α in a human gingival epithelial cell line, Ca9-22 (200). Studies on the expression of ICAM-1 in blood vessels (arterioles, venous and capillary ECs) of the dental pulp of patients with chronic periodontitis demonstrated a variable expression in control subjects and in patients with chronic periodontitis (194).

A number of investigations aimed to define effect of bacterial antigens (mainly those of *Porphyromonas gingivalis*) on expression of adhesion molecules (195,201,206–208). The pattern of ICAM-1 expression depended on the cellular model. Gingipains (cysteine proteases) from *P. gingivalis* exerted a direct proteolytic action on ICAM-1 expressed on cultured oral epithelial cells (KB and HSC-2) (206). As a consequence, the interaction of PMNs with oral epithelia would be disrupted and periodontal tissues would be damaged by bacteria (206). Using the KB line, other authors demonstrated colocalization of *P. gingivalis,* ICAM-1 and caveolin-1. Using goat polyclonal anti-ICAM-1 serum, it was possible to inhibit the infection of KB cells with *P. gingivalis*. According to these investigators, ICAM-1 plays a significant role at the first stages of development of periodontal disease, when bacteria attack epithelial cells. Decreased or no expression of ICAM-1 might inhibit infection of epithelial cells with *P. gingivalis* and slow down development of periodontal disease (207). Finally, the studies related to the expression of ICAM-1 on ECs showed that ligation of CD99 on ECs [via nuclear factor-kappaB (NF-κB) activation] induced expression of many adhesion molecules, including ICAM-1, and resulted in increased leukocyte adhesion (195). Following application of gingipains to ECs, a dose-dependent reduction of adhesion molecule expression and leukocyte adhesion was demonstrated, induced by the ligation of CD99 on ECs (195). Indirectly, this was believed to reflect a disruption of adhesion molecule expression and of leukocyte recruitment to inflammatory foci (195). Results contrasting with the above were obtained by Zhang *et al*. (201). Modification of ICAM-1 production in the endothelium of umbilical veins (cell line

ECV-304) by treatment with *P. gingivalis* strains of variable virulence provided evidence not for inhibition but for induction of ICAM-1 expression, slightly more pronounced in the case of a bacterial strain of a higher invasiveness. Moreover, the induction of ICAM-1 production was shown to occur with the involvement of, first of all, the NF-κB and not the p38 MAPK signaling pathway. ICAM-1 production in ECs was abrogated by inhibition of the NF-κB pathway (201). Recent studies in cultured mouse macrophages (RAW264.7) demonstrated increased production of ICAM-1 and LFA-1, as well as a significant role of these molecules, in plaque-like formation of periodontopathic *Aggregatibacter acinomycetemcomitans* lipopolysaccharide-stimulated macrophages. Such an effect suggested participation of ICAM-1 in promotion of atherogenesis by activating leukocytes in the course of periodontitis (208).

In peridontal diseases, similarly to systemic diseases, application of a therapy was suggested which blocks the function of ICAM-1, as it was shown that in such diseases endothelial expression of the molecule can be inhibited using antisense oligonucleotides (187). In recent years, studies have demonstrated that resveratrol decreased leukocyte (mainly monocyte) adhesion to ECs, induced by lipopolysaccharide from *P. gingivalis*, by decreasing expression of ICAM-1 and VCAM-1 along the NF-κB signaling pathway (209).

The results of studies on the remaining surface adhesion proteins linked to HEV endothelium (CD34, PECAM-1 and endoglin) indicate that these molecules served mainly to mark a phenotype of gingival blood vessels, but were not directly connected with disease progression. Expression of CD34 was demonstrated on the proliferating ECs of gingiva, including HECs in patients with chronic periodontitis (41,43). Immunohistochemical studies detecting this marker of ECs confirmed proliferation and reconstruction of blood vessels in gingiva encompassed by the inflammatory process (41,210). CD34 served also as a clinically useful marker of circulating, more mature, EPCs, the number of which manifested a positive correlation with chronic periodontitis progression, age, gender of the patient, concentration of C-reactive protein and carotid intima-media thickness (188). Vascular expression of CD34 was examined also in gingival hypertrophy, induced by cyclosporine A treatment in patients with liver transplantation. The number of CD34-positive gingival vessels was compared before and after nonsurgical periodontal therapy (211). Another approach to studies on CD34 expression in gingival tissues was presented by Ohta *et al.* (212). They showed that mesenchymal stem stells and HSCs expressing CD34 were not involved in the regeneration of the periodontium (212).

PECAM-1 represents one of the most abundant adhesion molecules in ECs, playing a key role in adhesion and in interactions with leukocytes (195). The available literature was found to contain individual investigations on expression of PECAM-1 and endoglin (CD105), mainly as markers of periodontitis (194,195,197). In contrast to ICAM-1, expression of PECAM-1 was demonstrated mainly on two types of gingival cells (lymphocytes and ECs) in patients with gingivitis and periodontitis. Expression of PECAM-1 on lymphocytes was similar in the two disease states but it increased significantly in proportion to the size of the inflammatory infitrate. The positive immunocytochemical reaction for PECAM-1 (apart from ICAM-1) was observed also in ECs, but the authors did not associate the presence of the protein with the occurrence of HECs in the tissues studied (197). Taşman *et al.* (194) failed to detect significant differences in intensity of PECAM-1 expression between the chronic periodontitis group and controls. Endoglin (CD105) and ICAM-1 were expressed at varying intensities in tissue biopsies of both groups.

Activated gingipains preferentially down-regulated the expression of PECAM-1 on ECs. Endothelial monolayers demonstrated progressive intercellular gap formation, which correlated with reduced expression of intercellular junctional PECAM-1. This was accompanied by an increased permeability of a single EC layer for albumin and neutrophils (195).

Attempts at therapeutic application of antibodies to adhesion molecules

Disturbances in expression or overexpression of adhesion molecules were observed in inflammatory and allergic processes as well as in malignant tumors of humans (148). Attempts to block the action of these molecules as a novel therapeutic approach were undertaken using *in vitro* models and in experimental animals as well as in humans (reviewed in 213,214). These studies used antibodies and antisense oligonucleotides against human adhesion molecules (213–222). Application of such therapy was implemented mainly in acute and chronic inflammatory states and in autoimmune diseases (108,213,222–225). Subsequently, this type of therapy was introduced to inhibit neoangiogenesis in solid tumors (126,221,226), hematologic diseases (215,216) and in interventional cardiology, most frequently to enhance re-endothelialization (217). Most descriptions of anti-adhesive therapy applications pertained to attempts to block ICAM-1 (213,214,222–225,227,228).

To date, no data are available on applications of anti-adhesion molecule therapy in periodontology.

Immunocytochemical evaluation of gingival membrane markers in HEVs in chronic periodontitis – our own studies

The dominant presence of HEVs in gingiva of patients with chronic periodontitis, and their absence in gingiva within clinically healthy periodontia, is well known (15,42,43,50,51). In our own studies, proliferation of various types of small blood vessels (mainly of capillaries and venules) was observed in chronic periodontitis. In our studies we decided to characterize periodontal HEVs with respect to expression of adhesion molecules with the most profound role in transendothelial migration and angiogenesis. It was

assumed that vascular expression of leukocyte adhesion molecules is a critical determinant of tissue response to microbial challenge in the pathogenesis of chronic periodontitis. The location of surface markers (CD34, PECAM-1, endoglin and ICAM-1) of ECs in the gingiva of 40 patients with chronic periodontitis was compared with an analogous expression in HEVs of reactive lymph nodes (positive control) and with ECs of blood vessels in the gingiva of patients with no periodontal pathology (negative control). Using the classical streptavidin-biotinylated peroxidase complex (ABC) technique and commercially available antibodies, distinct patterns of adhesion molecule expression were detected in all types of blood vessels. Application of a novel technique of spatial visualization permitted quantitative analysis of the immunocytochemical reaction for a given marker and for comparative studies (216).

In line with expectations, an endothelial location of the proteins prevailed. All the molecules were presented in gingival HEVs in patients with chronic periodontitis (Fig. 4A) and in HEVs of reactive lymph nodes (Fig. 4B), as well as in cell membranes of typical blood vessels (small arterioles, venules and capillaries) (Fig. 4C). CD34 was a classical marker of the luminal part of EC membranes in gingival blood vessels of patients with chronic periodontitis (Fig. 4A). Expression of PECAM-1 showed typical localization on lateral surfaces of HEC membranes, in the site of intercellular attachments (Fig. 4D), and, of note, on the cell membranes of extravascular leukocytes (mononuclear cells and individual neutrophils) of the inflammatory infiltrates (Fig. 4D). Quantitatively, in the gingival HEVs of patients with chronic periodontitis, PECAM-1 was most abundant and, in reducing order, CD34, ICAM-1 and endoglin (CD105). The significantly enhanced expression of PECAM-1 and CD34, compared with those of ICAM-1 and endoglin, is consistent with the literature (107,114). Probably, the augmented expression of PECAM-1 is connected with the angiogenesis process (111,112). The significantly higher total expression of the four membranous markers in gingival HEVs of the chronic periodontitis group, compared with the total expression in the control and the high positive reciprocal correlations among CD34, endoglin and ICAM-1 in gingival HEVs, may point to the role of these molecules in transendothelial migration in chronic periodontitis. Overexpression of the adhesion molecules in patients with chronic periodontitis compared with the control is of significance in protracted inflammatory processes and periodic exacerbations of the inflammatory process and the subsequent destruction of periodontal tissues (229). Within the chronic periodontitis group, studies demonstrated significantly higher expression of CD34, endoglin and ICAM in gingival HEVs compared with the expression of analogous markers in typical gingival blood vessels. We have shown that the levels of expression of CD34 in HEVs and of PECAM-1 in other blood vessels in the gingiva of patients with chronic periodontitis correlate significantly with the histological score (grading) of inflammation (229).

Consecutive studies showed that patients with chronic periodontitis manifest a higher angiogenetic index (endoglin/PECAM-1), compared with control gingiva,which may indicate an increased angiogenesis in periodontal tissue altered by inflammation (230). This observation is consistent with the literature, demonstrating involvement of endoglin and PECAM-1 in angiogenesis in cases of neoplastic lesions (112). Our studies demonstrated a quantitatively similar expression of all the membrane markers studied (except for PECAM-1) in the gingival HEVs of patients with chronic periodontitis and in HEVs of reactive lymph nodes, which indicates a functional similarity of HEVs in pathologically altered tissues (230). However, it has been intriguing that studies have failed to demonstrate significant differences in expression of any vascular marker, depending on the value of the clinical

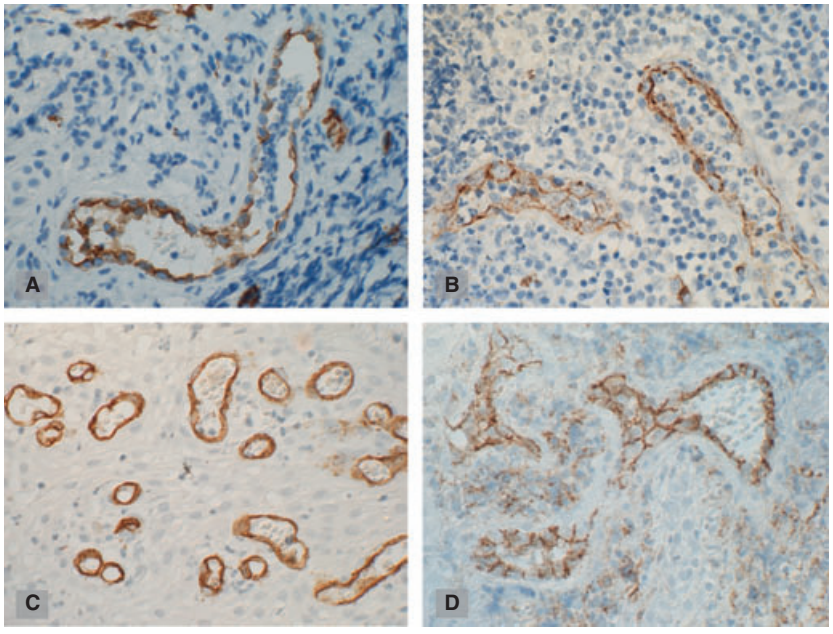

Fig. 4. Localization of selected adhesion molecules expressed by high endothelial cell postcapillary venules (HEVs) and blood vessels with flat endothelial cells. (A) CD34 expression in a fragment of the gingiva a patient with chronic periodontitis; (B) CD34 expression in a fragment of reactive lymph node; (C) CD34 expression in other gingival blood vessels of a patient with chronic periodontitis; (D) platelet endothelial cell adhesion molecule 1 (PECAM-1) localization in patients with chronic periodontitis. Note the characteristic localization (lateral aspects of the cell membranes) and some PECAM-1-positive inflammatory cells in the gingival lamina propria. Avidin-biotinylated peroxidase complex (ABC) technique. Hematoxylin counterstain. Objective magnification, ×40.

attachment level/loss (CAL) parameter, the form of the disease (local or generalized), and the age and sex of the patient with chronic periodontitis (230). Perhaps this reflects that we have studied material with a high prevalence of the most advanced inflammatory lesions (grading 3) and the most pronounced CAL parameter.

The putative role of surface adhesion molecules expressed by periodontal HECs in patients with chronic periodontitis is shown in Fig. 5.

Concluding remarks

Proliferation of gingival blood vessels and alteration in the phenotype of endothelial cells allow chronic periodontitis to be included in the category of vasoproliferative diseases. The pathogenetic mechanisms that lead to the proliferation of postcapillary venules with high endothelium (periodontal HEVs) in gingival tissues remain relatively unknown, as do their role in chronic periodontitis. The principal role of HEVs in physiology involves their participation in the recruitment, migration and recirculation of lymphocytes from blood to lymphatic organs and the initiation of an immune response following contact with antigen. The steps of leukocyte transendothelial migration in peripheral lymphatic organs are known in detail; and the directional movement of cells remains under the guidance of adhesion molecules. The typical glycoproteins on the surface of ECs are termed the addressins.

Few studies have been carried out on surface adhesion molecules associated with HEV endothelium, such as CD34, PECAM-1 and endoglin, in the development of periodontal diseases. These molecules have been used mainly as markers of gingival blood vessels. The largest number of studies on the role of local expression of adhesion proteins in the development and progression of periodontal diseases pertain to ICAM-1 (CD54). This marker has even been termed the inflammation

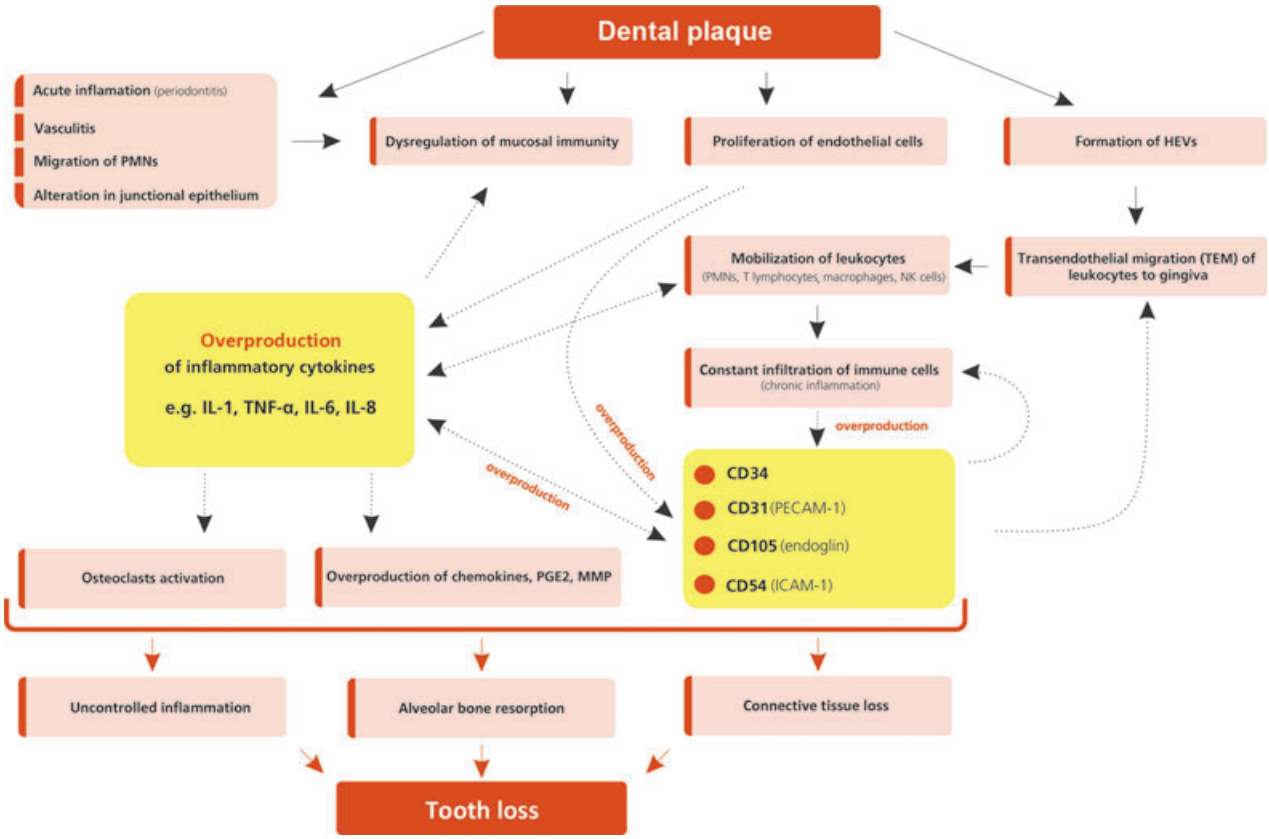

Fig. 5. Diagram demonstrating several of the key processes in the destruction of periodontal tissues, with involvement of the adhesion molecules [CD34, platelet endothelial cell adhesion molecule 1 (PECAM-1), endoglin and intercellular adhesion molecule 1 (ICAM-1)] expressed on endothelial cells (ECs) [including high endothelial cells (HECs)]. Chronic periodontitis is accompanied by the proliferation of small blood vessels in the gingiva and by the occurrence of specialized postcapillary high endothelial venules (HEVs). The suggested role of HEVs in the pathogenesis of chronic periodontitis involves their participation in the transmigration of different types of leukocytes [mainly polymorphonuclear leukocytes (PMNs)] to inflamed periodontium and in proinflammatory effects. The proinflammatory effects comprise overproduction of the cytokines, prostaglandin E2 (PGE2), MMPs, multiple classes of L-selectin ligands, leukocyte-specific chemokines, chemokine-binding proteins and junctional adhesion molecules by infiltrating leukocytes or endothelial cells (including HECs). The local expression of ICAM-1 (CD54) and PECAM-1 seems to play the most important role in the development and progression of periodontal diseases. For details see the main text. IL, interleukin; NK, natural killer; TNF-α, tumor necrosis factor-α.

biomarker of periodontitis and it is mainly this marker which is considered a potential therapeutic target in periodontal pathology.

Our studies have demonstrated highly positive reciprocal correlations among expression of CD34, endoglin and ICAM-1 in gingival HEVs, which may point to a role of these molecules in transendothelial migration, in chronic periodontitis. Local expression of ICAM-1, resembling expression of endoglin, was not high in the patients we studied with chronic periodontitis. Perhaps more extensive clinical significance could be linked to determination of the angiogenesis index (endoglin/PECAM-1), which reflects enhanced angiogenesis in inflamed periodontal tissues. However, determination of whether the biomarkers of gingival HEVs might be used to identify sites with an enhanced risk of progression of periodontal diseases and a better response for potential treatment requires continuation of the studies. According to recent data and our studies, local expression of ICAM-1 (CD54) and PECAM-1 seem to play the most important role in the development and progression of periodontal diseases, but much work remains to be conducted to understand in greater detail the mechanisms of the adhesion molecules in transendothelial migration and periodontal diseases.

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