Basic Res Cardiol 93: Suppl 3, 30 – 39 (1998)

H.-J. Schnittler

Structural and functional aspects of intercellular junctions in vascular endothelium

Abstract Cell-to-cell-junctions of endothelial cells are specialized and differentiated areas of the plasma membrane. The main functions include the separation of the intravascular and extravascular compartments, the mechanical connection of the cells, and the maintenance of the cell polarity. Although a wide heterogeneity of endothelial cell-to-cell junctions exists in situ, they should be considered in general as adherens type junctions in which gap and tight junctions are morphologically inserted.

Under certain pathological conditions, such as wound healing, angiogenesis and many types of inflammation, the interendothelial junctions have to be dissociated and reorganized in which proteins of the junctions are crucially involved. These important mechanisms predict a sophisticated regulation of junctional proteins. The present paper describes the organization and functional aspects of the occludin/ZO-1 complex typically found in tight junctions, the cadherin/catenin complex of the adherens junctions and the connection of these protein complexes to the dense peripheral band via actin filaments. In addition, special attention has been drawn on the function of junction-associated proteins with respect to their role under fluid shear stress and interendothelial gap formation during inflammation.

Key words VE-cadherin – catenins shear stress – permeability actin filaments – cytoskeleton

H.-J. Schnittler (✉)
Institute of Physiology
Robert-Koch-Straße 27a
D-48149 Münster, Germany
Email: schnith@uni-muenster.de

Introduction

The endothelium has to be considered as a wide spread tissue lining the entire surface of the heart and the vessels. It significantly contributes to a wide variety of cardiovascular functions, which include the regulation of permeability, blood pressure, coagulation, transmigration of leukocytes, exchange of oxygen, carbon dioxide, nutritients and metabolites. For proper function of the endothelium, integrity is required, i.e., mainly ensured by endothelial cell-to-cell junctions.

Morphologically, endothelial cell-to-cell junctions of the blood vessels primarily consist of an extended adherence junctional zone in which tight junctions and gap junctions are inserted (12, 66, 67). Adherens junctions are common to all endothelial cells, but tight and gap junctions appear to be graduated within different vascular segments (66, 67). Both, tight and gap junctions are frequent in endothelial cells of large vessels and arterioles. In capillaries and postcapillary venules tight junctions are staggered or interrupted, respectively, and gap junctions are diminished or even absent (66, 67). Whereas gap junctions form channels between adjacent cells allowing intercellular communication, the adherens type junctions mechanically hold the cells together and the tight junctions are responsible for sealing properties between adjacent cells (1, 57, 76). The notable heterogeneous appearance of the endothelium within different organs and vascular segments also reflects wide variations in endothelial functions (66, 67).

Pathophysiological reactions such as inflammation (change of paraendothelial permeability and extravasation of leukocytes), wound healing, and angiogenesis (both associated with migration and proliferation) cause a dissociation and rearrangement of endothelial cell-to-cell junctions. With respect to these pathophysiological reactions, changes in the molecular interactions between proteins of the cell-to-cell junctions are required to allow cell migration, proliferation and changes in paraendothelial permeability. Junction-associated compo-

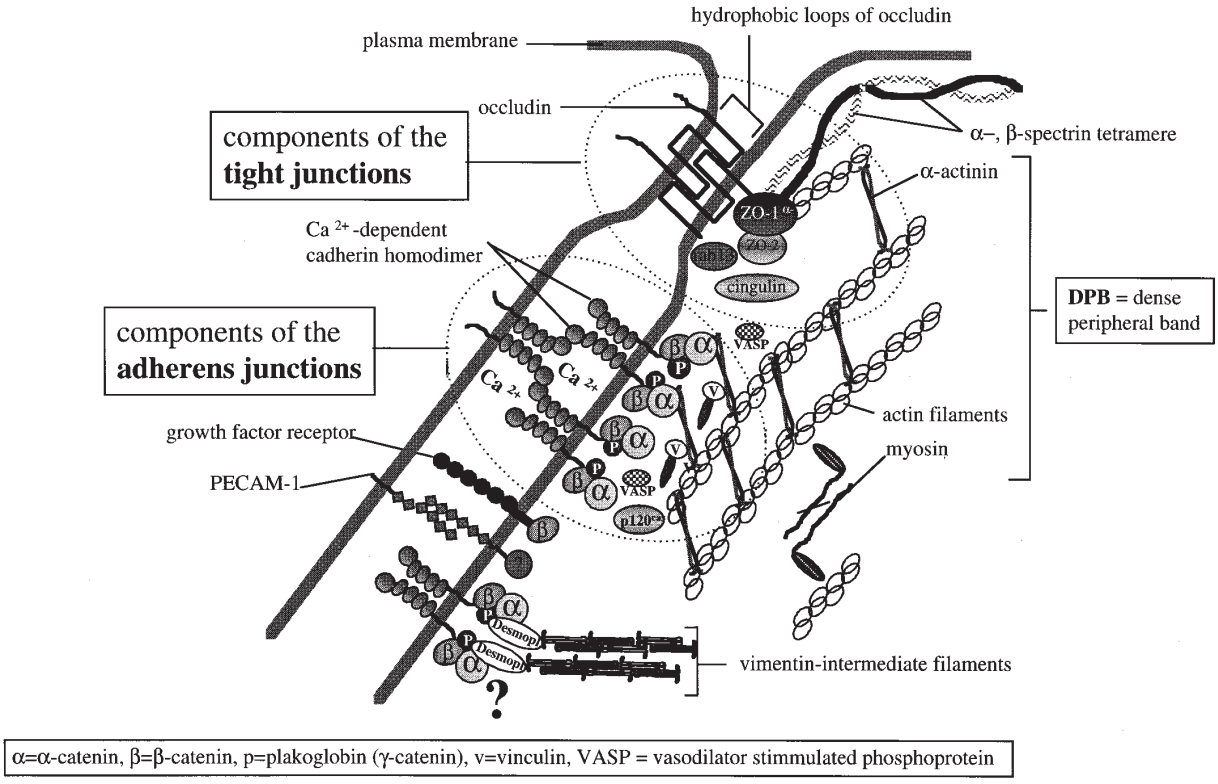

Fig. 1 Scheme illustrating the molecular organization of endothelial tight and adherens junctions (compare with Fig. 2). Adherens junctions are common to all endothelial junctions consisting of Ca^{2+} dependent cadherins, catenins, and others as illustrated. These protein complexes are connected to actin filaments that together with myosin filaments builds up the dense peripheral band (DPB) along the endothelial junctions. Tight junctions appear to be heterogeneous distributed in endothelial cells within the vascular system but its components, such as occludin and ZO-1, can be found. The DPB is a highly dynamic structure that might be involved in regulation of tight and adherens junctions. PECAM-1 a member of the Ca^{2+} independent immunoglobulin superfamily is also present at the junctions and is involved in transmigration of leukocytes. (Gap junctions and integrins are not included).

nents seem also to be involved in cellular signaling. For all these reasons, the molecular and functional analyses of endothelial cell-to-cell junctions are one of the primary subjects of current endothelial cell research.

Molecular organization of interendothelial junctions

The organization of cell-to-cell junctions is primarily mediated by integral membrane proteins (cell adhesion molecules) that display homophilic or heterophilic binding to integral membrane proteins (cadherins, occludin, PECAM-1, α_2/β_1 and α_5/β_1-integrins) of adjacent cells or extracellular matrix components. At the cytoplasmic face the integral cell adhesion molecules are connected to cytoskeletal filament systems (actin filaments, intermediate filaments) via intermediate proteins, such as catenins, α-actinin, ZO-1, ZO-2, desmoplakin, (for details see below and compare Figs. 1 and 2). The occurrence of such a wide variety of intermediate proteins might be related to specific and differentiated responses of the junctions to diverse stimuli. This assumption is supported by the observations that intercellular junctions are rich in signaling proteins of several signaling pathways (e.g., p100, $p120^{cas}$, rab 13, VASP, $p125^{FAK}$) and that components of junctions serve as protein kinase and protein-phosphatase substrates and contribute to transcriptional control (2, 3, 5, 10, 14, 17, 23, 25, 33, 44, 50, 65, 68, 69, 74, 75, 83, 85).

The tight and adherens junctional complexes are connected to the dense peripheral band (DPB) that is found at the cytoplasmic face along the junctional borders of adjacent endothelial cells in situ and in highly confluent endothelial cultures (Figs. 1 and 2) (16, 58, 79, 81). The DPB mainly consists of filamentous actin, myosin, and α-actinin (16, 58, 59, 79, 81) which provide the molecular machinery for generation of contractile force (9, 59). Immunoelectron microscopy of endothelial cells using antibodies directed to actin and myosin revealed an extension of the DPB from the upper part (apical

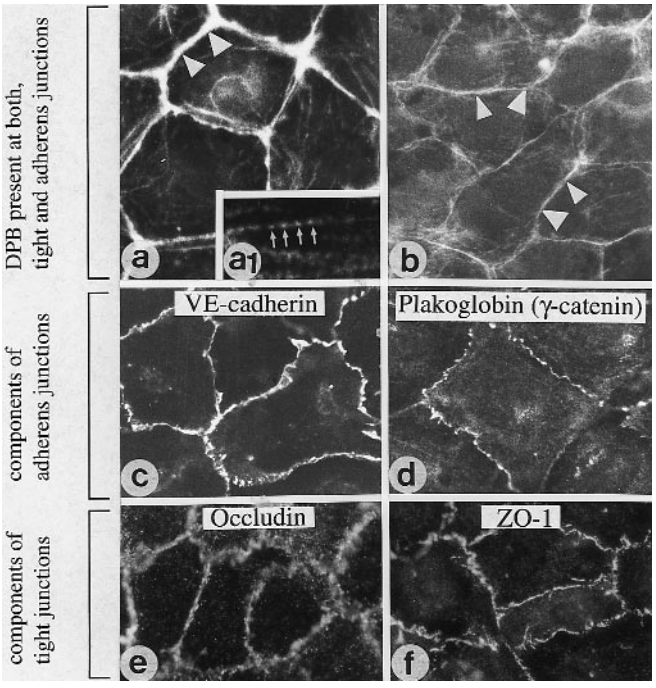

Fig. 2 Immunocytochemical localization of junction-associated proteins in endothelial cells of confluent cultures of the human umbilical vein (a, c, d, e, f) and of the right ventricle of the pig heart (b). Actin filaments (a, b) are marked with rhodamine labeled phalloidin (specifically labels actin filaments, but not actin monomers). Myosin (a1), VE-cadherin (c) plakoglobin (d), occludin (e), and ZO-1 (f) are labeled with appropriate antibodies. (a) Actin filaments are restricted to the margins of endothelial junctions (arrowheads) in highly confluent cultures of endothelial cells (a) and in low pressure segments of the cardiovascular system, here demonstrated for endocardial endothelial cells of the right ventricle of the pig heart in situ by confocal laser microscopy (b). Myosin is seen in a sarcomere-like pattern (a1), (arrows). Together with actin filaments, myosin provides the molecular base for contraction. Whereas VE-cadherin and plakoglobin (γ-catenin) are components of the adherens junctions, occludin and ZO-1 are typically components of tight junctons, although ZO-1 has also been described to occur in adhesion junctions. Note, in unstimulated endothelial cells, all proteins of the tight and adhesion junctions are localized in a continuous band along the endothelial borders.

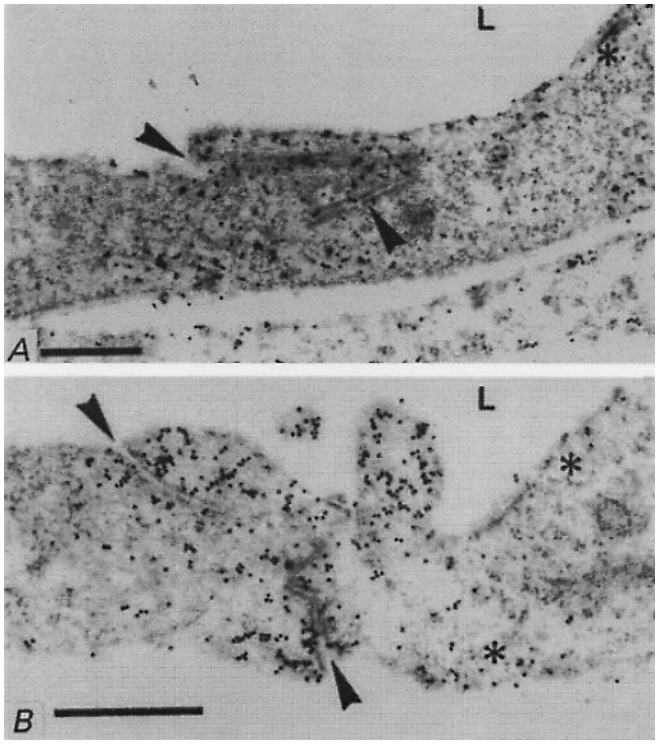

Fig. 3 Staining of actin (A) and myosin (B) at interendothelial junctions in endothelial cells of the rat skin by immunogold labeling (10 nm). Note, the immunoreaction of actin and myosin along the junctions from the apical pole down to the basement membrane. These structures are generally designated as dense peripheral band (DPB) and build a bridge between tight and adherens junctions that might be important for regulation of junctions under pathological conditions. Asterisks indicate labels close to the plasma membrane, (taken from 59).

cell surface) to the lower part (basal, close to the basement membrane) of the junctions (59) (Fig. 3). Evidence has been provided that connection of tight and adherens junctional complexes to actin filaments (component of the DPB) seems to be substantial for immobilization of the protein complexes and, thus, for junctional stability and function (for review see 73). The DPB is a highly dynamic structure which is considerably disintegrated after endothelial cell activation by proinflammatory agents and under wound healing conditions (16, 26, 38, 63, 71, 72, 79, 81, 82) and therefore might provide an regulatory link between the different types of cell-to-cell junctional proteins.

Endothelial tight junctions

Tight junctions in general are supposed to fulfill at least two functions. First, they contribute to the maintenance of cell polarity (1, 76), and second they seal adjacent cells inhibiting uncontrolled paracellular exchange of small molecules, macromolecules, and water (8, 57, 76, 78). However, the sealing properties of tight junctions vary between endothelial cells of different locations. The limited occurrence of endothelial tight junctions in postcapillary venules (66, 67) facilitates reabsorption of filtered fluids and might also be the reason for an increased permeability at these locations during inflammation (31, 41).

Occludin, a unique integral membrane protein appears exclusively at tight junctions in epithelial and non-activated endothelial cells in a continuous band (Figs. 1 and 2). Occludin does not display sequence homologies to other known cell adhesion molecules (15), and the presence of two hydrophobic extracellular loops that are nearly uncharged probably plays a central role in building and maintaining sealing fea-

tures of tight junctions. Based on sequence analysis, it has been assumed that the hydrophobic loops of adjacent cells bind in a homophilic interdigitating manner (for review see 1), and phosphorylation of occludin seems to play a central role in occludin adhesion (55). At the cytoplasmic face, occludin is directly or indirectly linked to the following proteins: the zonula occludens protein-1 and -2 (ZO-1, ZO-2) (20, 70), cingulin (7), the 7H6 antigen (not in endothelial cells) (84), and rab 13 (83). The molecular organization and functional role of these molecules in building, maintaining, and regulating tight junctions is beginning to be unraveled (for review see 1, 76). ZO-1 binds directly to the carboxyterminus of occludin and seems also to be associated with cadherin based adherens junctions as well as with spectrin a component of the membrane cytoskeleton (27). In contrast to epithelial tight junctions, endothelial cells express a ZO-1$^{\alpha-}$isoform, lacking 80 amino acids at the C-terminus (80). This isoform seems to be more plastic due to attachment to the underlying actin filament cytoskeleton than epithelial ZO-1. Such a plasticity might significantly influence the physiologically dynamic nature of endothelial tight junctions in response to pathological stimuli such as increased permeability in inflammation.

The detailed molecular organization of tight junctions is still unknown, but two models, the protein- and the lipid-model, have been hypothesized (78). The former is based on protein-protein interactions comparable to the organization of adherens type junctions. The high electrical resistance and the sealing properties of tight junctions in this model are thought to be mediated by interaction of the hydrophobic extracellular domains. The lipid model focuses on the presence of cylindrical inverted lipid micelles (assumed to be the tight junction strands) that are thought to be initiated and stabilized by occludin and associated proteins (57, 78). Experimental evidence supporting the lipid model has been obtained by determination of lateral diffusion of fluorescent phospholipids between adjacent cells in combination with a photobleaching technique (19, 78). Diffusion of phospholipids was demonstrated between adjacent cells that had developed tight junctions. This indicates a continuous phospholipid flow between at least the outer leaflets of the plasma membrane which is consistent with the lipid model of tight junctions (19, 78). In any case, tight junction formation and maintenance require occludin and occludin-associated proteins as well as the binding to the underlying actin filament system (40).

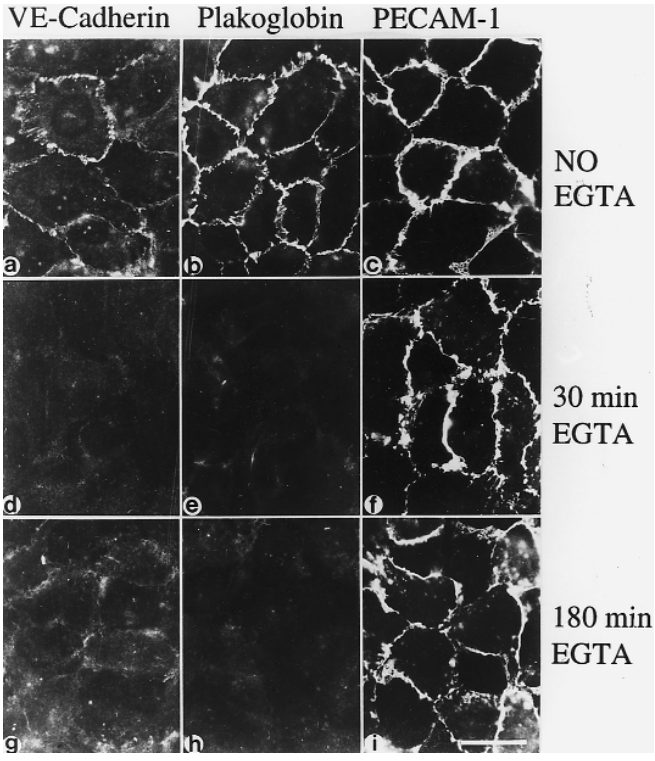

Fig. 4 Staining of confluent cultures of human umbilical vein endothelial cells with antibodies directed to vascular endothelial cadherin (VE-cadherin), plakoglobin (γ-catenin), and platelet endothelial cell adhesion molecule 1 (PECAM-1) before (a–c) and after (d–i) extracelluar Ca^{2+} depletion (addition of 3 mM EGTA resulting in a final concentration of < 10^{-7} M). Note the absence of VE-cadherin and plakoglobin after Ca^{2+}-depletion but PECAM-1 remains unchanged. Importantly, there is no dissociation of cell-to-cell junctions even after 180 min following Ca^{2+} depletion as indicated by the continuous staining of PECAM-1 (partially taken from 64).

Adherens junctions in endothelial cells

Adherens junctions mechanically connect endothelial cells and provide the structural base for interendothelial mechanical stability. Adherens junctions consist of integral membrane proteins that belong to the Ca^{2+} dependent cadherin family with the subtypes VE-cadherin (35, 36), P-cadherin (37), N-cadherin (58), and E-cadherin (54). At the cytoplasmic face, cadherins are associated with actin filaments of the DPB system via α-, β-, γ-actinin and α-actinin (25, 29, 32, 45, 52). Whereas β- and γ-catenin are connected directly to cadherins, α-catenin provides a link to the actin filament system via α-actinin (32) (Fig. 1). Further proteins, such as vinculin, $p120^{cas}$, and the vasodilator-stimulated phosphoprotein (VASP) are also localized along the cell-to-cell junctions (22, 86) and might be important to specific responses of the junctions to certain stimuli. A central role of the Ca^{2+} dependent cadherins and γ-catenin (plakoglobin) in mechanical stability of endothelial cells has been recently demonstrated (64). Depletion of extracellular Ca^{2+} to concentrations < 10^{-7} M caused a disapperance of both cadherins and catenins from cell-to-cell junctions, whereas the Ca^{2+} independent platelet endothelial cell adhesion molecule-1 (PECAM-1) remained unchanged (Fig. 4). Under resting conditions intercellular integrity was completely maintained, but the application of shear stress,

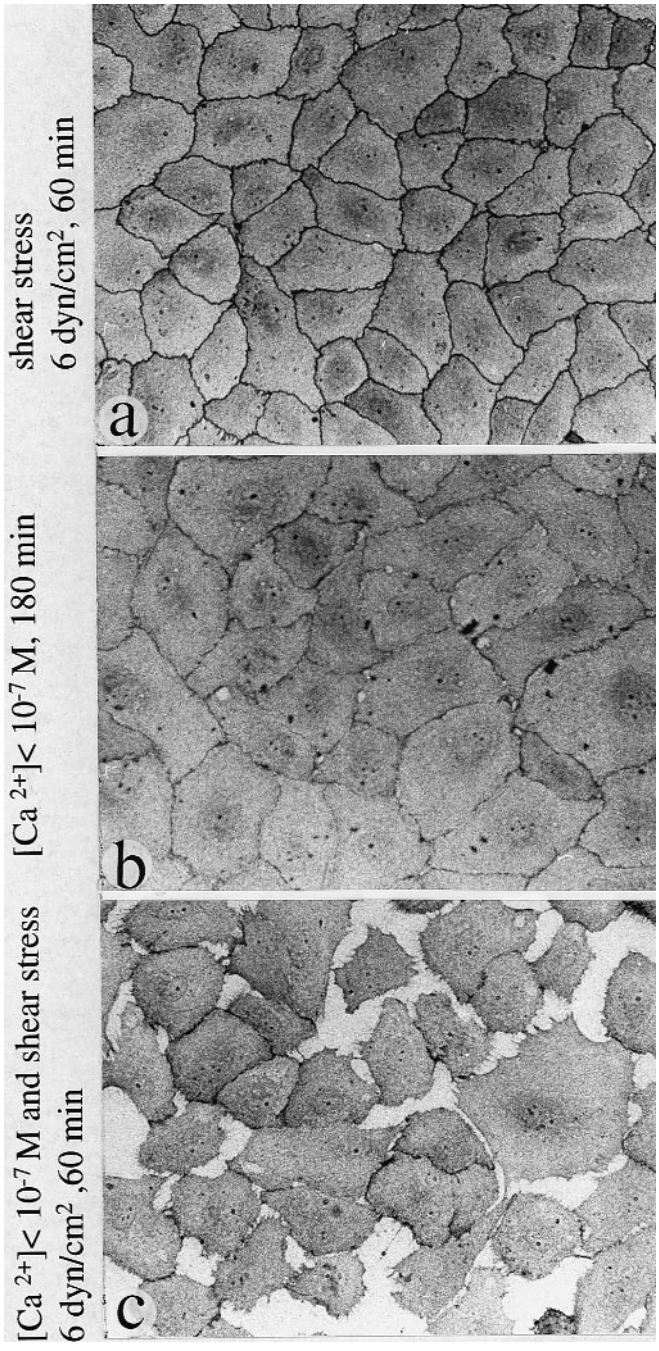

Fig. 5 Cultured human umbilical vein endothelial cells stained by sliver nitrate to visualize endothelial cell-to-cell junctions in Nomarsky optic under Ca^{2+} depletion and shear stress. Cells exposed to shear stress in normal Ca^{2+} concentration (a) or after Ca^{2+} depletion under resting conditions (b) did not display any intercellular gaps or disturbances of endothelial integrity. In contrast, Ca^{2+} depletion and shear stress (c) caused a dissociation of cell-to-cell junctions within minutes. These data indicate a central role of the Ca^{2+} dependent cadherin-cadherin interaction to ensure intercellular integrity under mechanical loads of fluid shear stress.

using a cone and plate rheological system (60), caused a marked dissociation of cell-to-cell junctions (Fig. 5). This shows a crucial role of the cadherin/catenin-complex in interendothelial cell adhesion in fluid shear stress and that PECAM-1 (independent from the cadherin-catenin-complex) is not able to compensate for the loss of cadherin-cadherin-interaction under these conditions. An important role of plakoglobin (γ-catenin) has been demonstrated in interendothelial adhesion under the load of fluid shear stress (64). Knock out of plakoglobin in cultured endothelial cells by microinjection of antisense oligonucleotides caused a depletion of plakoglobin in confluent cultures of endothelial cells within 24 h, but leaves cadherins, α-catenin, β-catenin, and PECAM-1 unaffected (64). Under resting conditions cell-to-cell-junctions were observed as a continuous uninterrupted structure, but the application of fluid shear stress caused interendothelial gap formation between plakoglobin depleted cells (64) (Fig. 6). The data indicate a crucial role of plakoglobin in maintaining

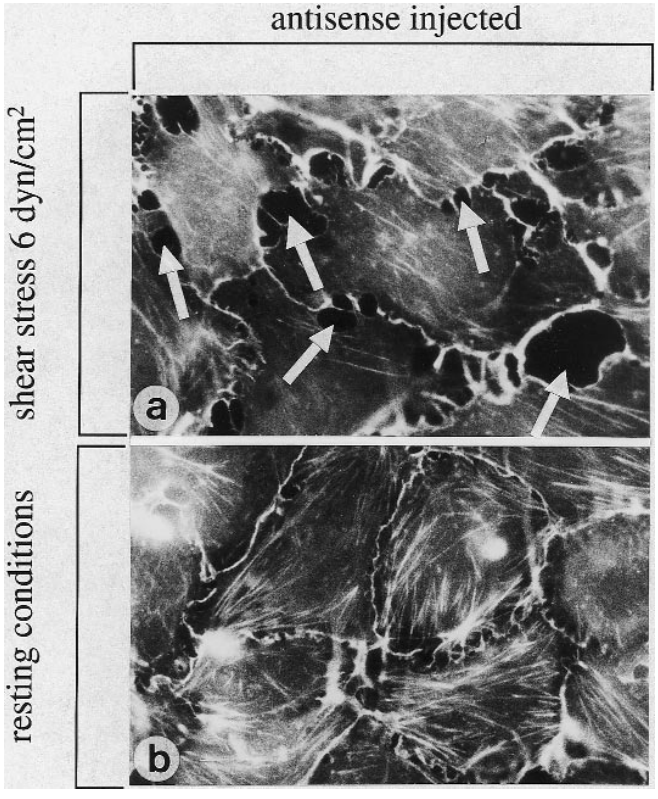

Fig. 6 Confluent cultures of porcine endothelial cells after depletion of plakoglobin by microinjection of antisense oligonucleotides (a, b) under resting conditions (b) and after exposure to fluid shear stress (a). Cells are stained with phalloidin-rhodamine to visualize interendothelial gap formation. Note, cells do not display intercellular gaps after plakoglobin depletion (b) but large gaps are visible (arrows) after shear stress of 6 dyn/cm². This indicates that plakoglobin is essential to maintain endothelial integrity under mechanical loads of fluid shear stress.

cell-to-cell junctional integrity under shear stress and further supports the hypothesis that plakoglobin might be essential in maturation of endothelial cell-to-cell junctions (36). Moreover, plakoglobin may contribute together with β-catenin to transcriptional control and gene regulation. This was concluded from the observation that both proteins are translocated into the nuclei in developing xenopus blastomers (10) and that β-catenin binds to transcription factor LEF-1 (3). In addition, β-catenin (compare Fig. 1) binds to growth factors (25) and has also been suggested to play a role in translocation of ZO-1 to tight junctional complexes (49).

Recently a further adherens junction-associated protein, desmoplakin, was identified in interendothelial junctions in cultured human umbilical vein endothelial cells (77). Desmoplakin is a well-known component of desmosomes in epithelial cells and is linked to keratin intermediate filaments (for review see 13). However, vascular endothelial cells in general do neither express keratin (for exceptions see 28) nor do they display desmosoms. Therefore, the presence of desmoplakin at endothelial junctions might be important to link endothelial intermediate filaments of the vimentin-type (typical intermediate filaments of the vascular endothelium) to the junctions and subsequently may contribute to endothelial and interendothelial mechanical stability.

Further junction-associated proteins

A special feature of interendothelial junctions is the presence of platelet endothelial cell adhesion molecule-1 (PECAM-1), a member of the Ca^{2+} independent immunoglobulin superfamily (compare Figs. 1 and 4). PECAM-1 is also expressed in platelets, monocytes, neutrophils, and some T-cells (for review see 8a) but is not found in epithelial junctions. It is probably involved in mediating inflammatory responses such as monocyte-endothelial interactions. Moreover, PECAM-1 is a ligand for α_v/β_3-integrin (47), which is known to be involved in angiogenesis (4). α2/β1 and α5/β1 integrins have been shown to be localized at interendothelial junctions as well (34) and may also be involved in angiogenesis. These proteins are members of the integrin superfamily that typically mediate cell-substrate adhesion of adherent cells and might serve as signal recognition molecules as known from various integrins. The specific role of integrins at interendothelial junctions is still unknown.

Functional aspects of tight and adherens junctions in endothelial permeability

Increased endothelial permeability during inflammation can be caused by certain agents, such as histamine, thrombin, bradykinine, serotonin, cytokines, peoxides, prostacyclins (38). Whereas histamine and thrombin cause a quick increase in endothelial permeability, polypeptide mediators, such as cytokines, are long lasting and require de novo and/or increased transcription as well as protein synthesis (38). It should be noted that increase in permeability caused by cytokines seems to be the result of various, in concert acting mediators that are released from cells of the mononuclear phagocyte system (MPS) and other leukocytes after stimulation (e.g., by bacteria, viruses, parasites, cytokines). Toxins, such as clostridium botulinum C2- and clostridium difficile C3-toxin and E. coli hemolysin, similarly increase endothelial permeability (24, 71, 72). All these agents act via different signaling mechanisms, but the final result is the local dissociation of tight and adherence junctions (11) and a reorganization of the actin-containing microfilament systems (16, 26, 38, 46, 63, 71, 72, 79, 81, 82). Although formation of transcellular holes in frog mesenteric capillaries have been described after stimulation with the Ca^{2+} ionophore A 23187 (42) or application of VEGF (53), increased permeability in situ and in cultured endothelial cells is mainly correlated with formation of gaps between adjacent endothelial cells (for review see 38).

Activation of endothelial cells by thrombin caused dissociation of intercellular junctions (gap formation) (38) and was shown to be accompanied with phosphorylation of β-catenin and γ-catenin (48). Proteinkinase C inhibitors prevented the phosphorylation of β-catenin and γ-catenin and inhibited increase in permeability (48). This suggests that catenins are activated by phosphorylation, which seems to be critical for junctional stability and integrity. In addition, an increase in permeability provoked by supernatants of virus activated macrophages or recombinant human TNF-α in the presence of H_2O_2 (10 μM) was associated with interendothelial gap formation and caused redistribution of the adherens junction-associated protein VE-cadherin, α-catenin, β-catenin, plakoglobin (γ-catenin), and PECAM-1 in cultured human umbilical vein endothelial cells (11, 61, 62) (Fig. 7).

Both, tight and adherens junctional complexes (see above) are linked to actin filaments of the DPB. Such a linkage might be important under circumstances when both tight and adherens junctions have to be cleaved (e.g., interendothelial gap formation caused by proinflammatory mediators, tumor cell extravasation, diapedesis of leukocytes). It has been shown that gap formation in general is associated with both, the dissociation of the DPB and a reorganization of actin filaments and associated proteins (16, 26, 38, 63, 71, 72, 79, 81, 82). Actin filaments are regulated by Rho proteins, small GTP-binding proteins, that are probably the top of several signaling cascades (21, 39, 43). Whereas activation of Rho is required to cadherin-dependent formation of cell-to-cell junctions in epithelial cells (6), inactivation of Rho proteins plays a critical role in interendothelial gap formation and is associated with increase in endothelial permeability (24). This has been

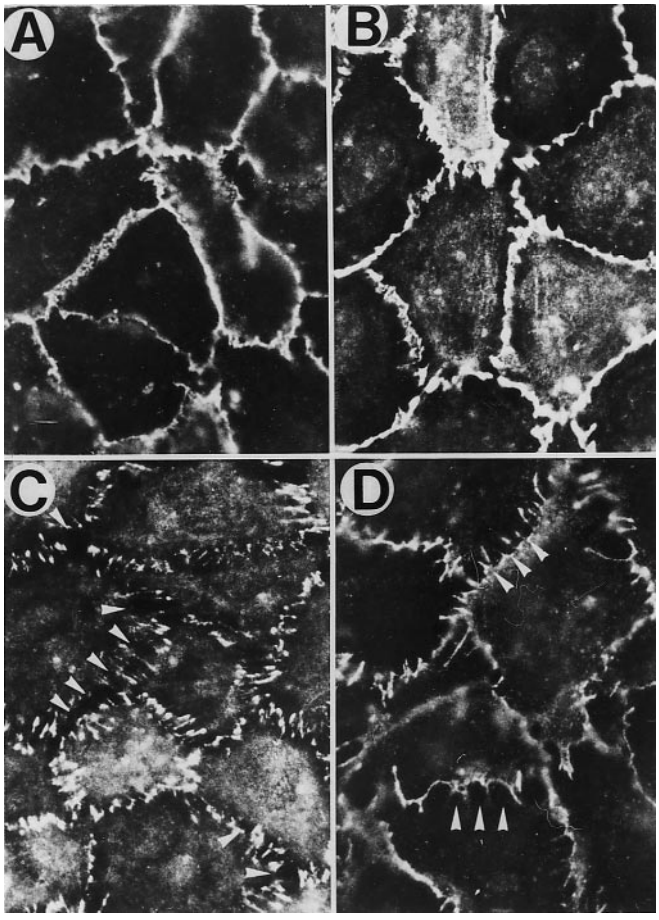

Fig. 7 Visualization of plakoglobin redistribution following stimulation with culture supernatants of filovirus activated monocytes/macrophages. Culture supernatants of activated monocytes/macrophages containing proinflammatory agents such as TNF-α. (A) A continuous band of plakoglobin immunoreactivity is seen in untreated cultures and (B) in cultures treated with culture supernatants taken from uninfected monocytes/macrophages. (C) Gap formation and redistribution of plakoglobin (arrowheads) is seen in endothelial cultures treated with culture supernatants of filovirus activated monocytes/macrophages. Under these conditions an increase in paraendothelial permeability was measured; for details see (8). (D) A redistribution of junction-associated proteins was also observed after treatment of endothelial cultures with recombinant TNF-α (10 ng/ml) and H_2O_2 (10 µM). These phenomena demonstrate that the increase of endothelial permeability is associated with a redistribution of junction-associated-proteins. Fig. 7 was reproduced from (8).

demonstrated using clostridium difficile toxin B that inactivates Rho by glycosylation at threonine 37 (30). Clostridium difficile toxin B induced permeability could not be blocked by elevation of cyclic nucleotides which act as earlier signaling molecules (24). Rho proteins also activate a tyrosine kinase (51) that in turn causes phosphorylation of several proteins including $pp125^{FAK}$ (51), which has been shown to be localized at focal contacts and cell-to-cell junctions (74). Thus, actin filament dynamics might be modulated via cell-to-cell junction-associated proteins. This is consistent with cell signaling responses localized at the junctions. On the other hand, if preexisting actin filaments are directly depolymerized, a dissociation of cell-to-cell junctions can be observed. This has been shown in cultured endothelial cells by application of clostridium botulinum C2-toxin (72). Clostridium botulinum C2-toxin is highly specific to ADP-ribosylate actin monomers (ADP-r-actin) and, therefore, causes actin filament net depolymerization and in turn increases endothelial permeability by interendothelial gap formation (72). The same effect could be directly obtained after microinjection of ADP-r-actin in endothelial cells. Under these conditions indirect effects of actin filament alteration can be largely excluded and show that actin filaments are not only essential in endothelial cells for functionally competent interendothelial junctions (unpublished observation).

Evidence has been provided that also contraction of endothelial cells is involved in interendothelial gap formation. In confluent cultures of skinned endothelial cells it has been shown that contraction, mediated by an actin-myosin-filament sliding mechanism, can be inhibited in endothelial cells using N-ethylmaleimide-modified S1-cross bridges (S1-NEM) prepared from skeletal muscle myosin. S1-NEM binds to actin filaments like the whole myosin molecule but is not able to dissociate in the presence of ATP and Ca^{2+}. Incubation of S1-NEM to skinned endothelial cells inhibited interendothelial gap formation (58, 59) which suggests that actin-myosin filament sliding mechanism is involved in interendothelial gap formation. In addition, it has been shown that gap formation induced by proinflammatory agents requires myosin light chain phosphorylation (18, 59, 82) which is a prerequisite for contraction in smooth muscle and non muscle cells. These results suggest a contraction of endothelial cells as a mechanism to cause gap formation. It has to be verified if contraction or microfilament reorganization or both is critical in interendothelial gap formation before or after activation of cell-to-cell junctional proteins.

Acknowledgment This work was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 355 „Pathophysiologie der Herzinsuffizienz“ of the University of Würzburg, Germany).

References

1. Anderson JM, van Itallie CM (1995) Tight junctions and the molecular basis for regulation of paracellular permeability. Am J Physiol 269: G467–G475
2. Balda MS, Gonzalez-Mariscal L, Matter K, Cereijido M, Anderson JM (1993) Assembly of tight junction: The role of diacylglycerol. J Cell Biol 123: 193–302
3. Behrens J, von Kries JP, Kühl M, Bruhn L, Wedlich D, Grosscheld R, Birchmeier W (1996) Functional interaction of β-catenin with the transcription factor LEF-1. Nature 382: 638–642
4. Bischoff J (1995) Approaches to studying cell adhesion molecules in angiogenesis. Nature 5: 69–74
5. Brady-Kalnay S, Rimm DL, Tonks NK (1995) Receptor protein tyrosine phosphatase PTPμ associates with cadherins and catenins in vivo. J Cell Biol 130: 977–986
6. Braga VMM, Machesky LM, Hall A, Hotchin NA (1997) The smal GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. J Cell Biol 137: 1421–1431
7. Citi S, Sabanay H, Jakes R, Geiger B, Kendrick-Jones J (1988) Cingulin, a new peripheral component of tight junctions. Nature 333: 272–276
8. Dejana E, Corada M, Lampugnani MG (1995) Endothelial cell-to-cell junctions. FASEB J 9: 910–918

8a. De Lisser HM, Newman RJ, Albelda SM (1994) Molecular and functional characterization of PECAM-1/CD31. Immunology Today 15: 490–49

9. Drenckhahn D, Wagner J (1986) Stress fibers in the splenic sinus endothelium in situ: molecular structure, relationship to the extracellular matrix, and contractility. J Cell Biol 102: 1738–1747
10. Fagotto F, Funayama N, Glück U, Gumbiner B (1996) Binding to cadherins antagonizes the signaling activity of β-catenin during axis formation in xenopus. J Cell Biol 132: 1105–1114
11. Feldmann H, Bugany H, Mahner F, Klenk HD, Drenckhahn D, Schnittler HJ (1996) Filovirus-induced endothelial leakage triggered by infected monocytes/macrophages. J Virol 70 (4): 2208–2214
12. Franke WW, Cowin P, Grund C, Kuhn C, Kapprell HP (1988) The endothelial junction. The plaque and its components. In: Simionescu N, Simionescu M (eds) Endothelial Cell Biology in Health and Disease. Plenum Publishing Corp. New York, pp 147–166
13. Fuchs E, Weber K (1994) Intermediate filaments: Structure dynamics function and disease. Annu Rev Biochem 63: 345–382
14. Funayama N, Fagotto F, McCrea P, Gumbiner B (1995) Embrionic axis induction by the armadillo repeat domain of β-catenin: evidence for intracellular signaling. J Cell Ciol 128: 959–968
15. Furuse M, Hirase T, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S (1993) Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol 123: 1777–1788
16. Gabbiani G, Gabbiani F, Lombardi D, Schwartz SM (1983) Organization of actin cytoskeleton in normal and regenerating arterial endothelial cells. Proc Natl Acad Sci USA 80: 2361–2364
17. Gebbink MFBG, Zondag GCM, Koningstein GM, Feiken E, Wubbolts RW, Moolenaar WH (1995) Cell surface expression of receptor protein tyrosine phosphatase RPTPμ is regulated by cell-cell contact. J Cell Biol 131: 251–260
18. Goeckeler ZM, Wysolmerski RB (1995) Myosin light chain kinase-regulated endothelial cell contraction: The relationship between isometric tension, actin polymerization, and myosin phosphorylation. J Cell Biol 130: 613–627
19. Grebenkämper K, Galla HJ (1994) Translational diffusion measurements of a fluorescent phospholipid between MDCK cells support the lipid model of the tight junctions Chem Phys Lipids 71: 133–143
20. Gumbiner B, Lowenkopf T, Apatira D (1991) Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. Proc Nat Acad Sci USA 88: 3460–3464
21. Hall A (1994) Smal GTP binding proteins and the regulation of the actin cytoskeleton. Ann Rev Cell Biol 82: 643–653
22. Haffner C, Jarchau T, Reinhart M, Hoppe J, Lohmann SM, Walter U (1995) Molecular cloning, structural analysis and functional expression of the proline-rich focal adhesion and microfilament-associated protein VASP. EMBO 14: 1–27
23. Hamaguchi M, Matsuyoshi N, Ohnishi Y, Gotoh B, Takeichi M, Nagai Y (1993) $p60^{v\text{-}src}$ causes tyrosine phosphorylation and inactivation of the N-cadherin-catenin cell adhesion system. EMBO J 12: 307–314
24. Hippenstiel S, Tannert-Otto S, Vollrath N, Krüll M, Just I, Aktories K, v Eichel Streiber C, Suttorp N (1997) Glucosylation of small-GTP binding rho proteins disrupt endothelial barrier function. Am J Physiol 272 (Lung Cell Mol Physiol 16): L38–L43
25. Hoschuetzky H, Aberle H, Kemler R (1994) Beta-catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. J Cell Biol 127: 1375–1380
26. Hüttner I, Walker C, Gabbiani G (1985) Aortic endothelial cell during regeneration. Remodeling of cell junctions, stress fibers, and stress fiber-membrane attachment domains. Lab Invest 53: 287–302
27. Ioh M, Yonemura S, Nagafuchi A, Tsukita S, Tsukita S (1991) A 220 kDa undercoat-constitutive protein: Its specific localization at cadherin based cell-to-cell adhesion sides. J Cell Biol 115: 1440–1452
28. Jahn L, Fouquet B, Rohe K, Franke WW (1987) Cytokeratins in certain endothelial and smooth muscle cells of two taxonomically distant vertebrate species, Xenopus laevis and man. Differenciation 36: 234–254
29. Jou TS, Stewart DB, Stappert J, Nelson WJ, Marrs JA (1995) Genetic and biochemical dissection of protein linkages in the cadherin-catenin complex. Proc Natl Acad Sci USA 92: 5067–5071
30. Just I, Fritz G, Aktories K, Giry M, Popoff MR, Boquet P, Hegenbarth S, von Eichel Streiber C (1995) Nature Lond 375: 500–503
31. Karnovsky MJ (1967) The ultrastructure basis of capillary permeability. J Cell Biol 35: 213–236
32. Knudsen KA, Peralta Soler A, Johnson KR, Wheelock MJ (1995) Interaction of α-actinin with the cadherin/catenin cell-cell adhesion complex via α-catenin. J Cell Biol 130: 67–77
33. Krypta RM, Su H, Reichardt LF (1996) Association between a transmembrane protein tyrosine phosphatase and the cadherin-catenin complex. J Cell Biol 134: 1519–1529
34. Lampugnani MG, Resnati M, Dejana E, Marchisio PC (1991) The role of integrins in the maintenance of endothelial monolayer integrity. J Cell Biol 112: 479–490
35. Lampugnani MG, Resnati M, Raiteri M, Pigott R, Pisacane A, Houen G, Ruco P, Dejana E (1992) A novel endothelial-specific membrane protein is a marker of cell-cell contacts. J Cell Biol 118: 1511–1522
36. Lampugnani M, Corada GM, Caveda L, Breviario F, Ayalon O, Geiger B, Dejana E (1995) The molecular organisation of endothelial cell to cell junctions: Differential association of plakoglobin, β-catenin, and α-catenin with vascular endothelial cadherin (VE-cadherin). J Cell Biol 129: 203–217

37. Liaw CW, Cannon C, Power MD, Kiboneka PK, Rubin LL (1990) Identification and cloning of two species of cadherins in bovine endothelial cells. EMBO J 9: 2701–2708
38. Lum H, Malik AB (1994) Regulation of vascular endothelial barrier function. Am J Physiol 11: L223–L241
39. Machesky LM, Hall A (1996) Rho: A connection between membrane receptor signalling and the cytoskeleton. Trends Cell Biol : 304–310
40. Madara JL, Barenberg D, Carlson S (1986) Effect of cytochalasin D on occluding junctions of intestinal absorptive cells: Further evidence that the cytoskeleton may influence paracellular permeability and junctional charge selectivity. J Cell Biol 102: 2125–2136
41. Majno G, Palade GE (1961) Studies on inflammation; I. The effect of histamine and serotonin on vascular permeability: An electron microscopic study. J Biophys Biochem Cytology 11: 571–626
42. Neal CR, Michel CC (1995) Transcellular gaps in microvascular walls of frog and rat when permeability is increased by perfusion with the ionophore A23187. J Physiol (Lond) 488 (2): 427–437
43. Nobes CD, Hall A (1997) Rho, rac and cdc 42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell 81: 53–62
44. Noordermeer J, Klingensmith J, Perrimon N, Nusse R (1994) Dishevelled and armadillo act in the wingless signalling pathway in drosophila. Nature 367: 80–83
45. Ozawa M, Baribault H, Kemler R (1989) The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. EMBO J 8: 1711–1717
46. Palade GE, Simionescu M, Siminoescu N (1979) Structural aspects of the permeability of the microvascular endothelium. Acta Physiol Scand (Suppl) 463: 11–33
47. Piali L, Hammel P, Uherek Ch, Bachmann F, Gisler RH, Dunon D, Imhof BA (1995) CD31/PECAM-1 is a ligand for α_v/β_3-integrin involved in adhesion of leuocytes to endothelium. J Cell Biol 130: 451–460
48. Rabiet MJ, Plantier JL, Rival Y, Genaux Y, Lampugnani MG, Dejana E (1996) Thrombin-induced increase endothelial permeability is associated with changes in cell-to-cell junction organization. Arterioscl Thromb Vasc Biol 16: 488–496
49. Rajasekaran AK, Hojo M, Huima T, Rodriguesz-Boulan E (1996) Catenins and Zonula occludens-1 form a complex during early stages in the assembly of tight junctions. J Cell Biol 132: 451–463
50. Renolds AB, Daniel J, McCrea PD, Wheelock MJ, Wu J, Zhang Z (1994) Identification of a new catenin: The tyrosine kinase substrate $p120^{cas}$ associates with E-cadherin complex. Mol Cell Biol 14: 8333–8342
51. Ridley JA, Hall A (1994) Signal transduction pathway regulating rho-mediated stress fiber formation: Requirement for a tyrosine kinase. EMBO 13: 2600–2610
52. Rimm DL, Koslov ER, Kebriaei P, Cianci CD, Morrow JS (1995) α_1(E)-Catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. Proc Natl Acad Sci USA 92: 8813–8817
53. Roberts WG, Palade GE (1995) Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J Cell Sci 108: 2369–2379
54. Rubin LL, Hall DE, Porter S, Barbu K, Cannon C, Horner HC, Janatpour M, Liaw CW, Manning K, Morales J, Tanner LI, Tomaselli KJ, Bard F (1991) A cell culture model of the blood-brain barrier. J Cell Biol 115: 1725–1735
55. Sakakibara S, Furuse M, Saitou M, Ando-Akatsuka Y, Tsukita S (1997) Possible involvement of phosphorylation of occludin in tight junction formation. J Cell Biol 137: 1393–1401
56. Salomon D, Ayalon O, Patel-King R, Heynes RO, Geiger B (1992) Extra junctional distribution of N-cadherin in cultured human endothelial cells. J Cell Sci 102: 7–17
57. Schneeberger EE, Lynch RD (1992) Structure, function, and regulation of cellular tight junctions. Am J Physiol 262: L647–L661
58. Schnittler H-J, Franke RP, Drenckhahn D (1989) Role of the endothelial actin filament cytoskeleton in rheology and permeability. Z Kardiol 78: Supp 6, 1–4
59. Schnittler HJ, Wilke A, Gress T, Suttorp N, Drenckhahn D (1990) Role of endothelial actin and myosin in the control of paracellular permeability. J Physiol 431: 379–401
60. Schnittler HJ, Franke RP, Akbay U, Mrowietz C, Drenckhahn D (1993) An improved in vitro rheological system for studying the effect of fluid shear stress on cultured cells. Am J Physiol 265 (Cell Physiol 34): C289–C298
61. Schnittler HJ, Feldmann H (1997) Filovirus-induced increase in endothelial permeability. Congress of molecular medicine Berlin: 3–5 May
62. Schnittler HJ, Feldmann H (1997) Molecular pathogenesis of filovirus infections: role of marcophages and endothelial cells. Curr Topics Microbiol Immunol (in press)
63. Schnittler HJ, Just I, Aktories K (submitted) Turnover of actin filaments in shear stress exposed endothelial cells and their role for cell integrity and adhesion
64. Schnittler HJ, Püschel B, Drenckhahn D (1997) Role of cadherins and plakoglobin in interendothelial adhesion under resting conditions and shear stress. Am J Physiol 273 (Heart and Circ Physiol 42): H2396–H2405
65. Shibamoto S, Hayakawa M, Takeuchi K, Hori T, Miyazawa K, Kitamura N, Johnson KR, Wheelock MJ, Matsuyoshi N, Tacheichi M, Ito F (1995) Association of p120, a tyrosine kinase substrate with E-cadherin/catenin complex. J Cell Biol 128: 949–957
66. Simionescu M, Simionescu N, Palade GE (1975) Segmental differentiations of cell junctions in the vascular endothelium. J Cell Biol 67: 863–885
67. Simionescu M, Simionescu N, Palade GE (1976) Segumental differentiations of cell junctions in the vascular endothelium. Arteries and Veins. J Cell Biol 68: 705–723
68. Staddon JM, Smales C, Schulze C, Esch FS, Rubin LL (1995) p120, a p120-related protein (p100), and the cadherin/catenin complex. J Cell Biol 130: 369–381
69. Staddon JM, Herrenknecht K, Smales C, Rubin LL (1997) Evidence that tyrosine phosphorylation may increase tight junction permeability. J Cell Sci 108: 609–690
70. Stevenson BR, Siliciano JD, Mooseker MS, Goodenough DA (1986) Identification of ZO-1: A high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J Cell Biol 103: 755–766
71. Suttorp N, Flöer B, Schnittler HJ, Seeger W, Bhakdi S (1990) Effects of Escherichia coli hemolysin on endothelial cell function. Infec Immun 58: 3796–3801
72. Suttorp N, Polley M, Seybold J, Schnittler HJ, Aktories K (1991) Adenosine diphosphate-ribosilation of G-actin by botulinum C2 toxin increases endothelial permeability in vitro. J Clin Invest 87: 1575–1584
73. Takeichi M (1990) Cadherins: A molecular family important in selective cell-cell adhesion. Ann Rev Biochem 59: 237–252

74. Tani T, von Koskul H, Virtanen I (1996) Focal adhesion kinase pp125^{FAK} is associated with both intercellular junctions and matrix adhesion sites in vivo. Histochem Cell Biol 105: 17–25
75. Tsukita S, Itoh M, Nagafuchi A, Yonemura S, Tsukita S (1993) Submembranous junctional plaque protzeins include potential tumor supressor molecules. J Cell Biol 123: 1049–1053
76. Tsukita S, Furuse M, Itoh M (1996) Molecular dissection of tight junctions. Cell Struct Funct 21 (5): 381–385
77. Valiron O, Chevrier V, Ussus Y, Breviario F, Job D, Dejana E (1996) Desmoplakin expression and organization at human umbilical vein endothelial cell-tocell junctions. J Cell Sci 109: 2141–2149
78. Wegener J, Galla HJ (1996) The role of non-lamellar lipid structures in the formation of tight junctions. Chem Phys Lipids 81: 239–255
79. White GE, Gimbrone Jr MA, Fujiwara K (1983) Factors influencing the expression of stress fibers in vascular endothelial cells in situ. J Cell Biol 97: 416–424
80. Willot E, Balba MS, Heintzelman M, Jameson B, Anderson JM (1992) Localization and differential expression of two isoforms of tight junction protein ZO-1. Am J Physiol 262: C1119–C1124
81. Wong AJ, Pollard TD, Herman IM (1983) Actin filament stress fibers in vascular endothelial cells in vivo. Science 219: 867–869
82. Wysolmerski RB, Lagunoff D (1990) Involvement of myosin light-chain kinase in endothelial cell retraction. Proc Natl Acad Sci USA 87: 16–20
83. Zahraoui A, Joberty G, Arpin M, Fontaine JJ, Hellio R, Tavitian A, Louvard D (1994) A small rab GTPase is distributed in cytoplasmic vesicles in nonpolarized cells but colocalizes with tight junction marker ZO-1 in polarized epithelial cells. J Cell Biol 124: 101–105
84. Zhong Y, Saitoh T, Minase T, Sawada N, Enomoto K, Mori M (1993) Monoclonal antibody 7H6 reacts with a novel tight junction-associated protein distinct from ZO-1, cingulin and ZO-2. J Cell Biol 120: 477–483
85. Zondag GCM, Moolenaar WH, Gebbink MFBG (1996) Lack of association between receptor protein tyrosine phosphatase RPTPμ and cadherins. J Cell Biol 134: 1513–1517
86. Yamada KM, Geiger B (1997) Molecular interactions in cell adhesion complexes. Curr Opin Cell Biol 9: 76–85