The parting of the endothelium: miracle, or simply a junctional affair?

Johnson-Leger, Caroline; Aurrand-Lions, Michel; Imhof, Beat

Journal of Cell Science 113, 921-933 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JCS0710

COMMENTARY

The parting of the endothelium: miracle, or simply a junctional affair?

Caroline Johnson-Léger, Michel Aurrand-Lions and Beat A. Imhof

Department of Pathology, Centre Médical Universitaire, 1211 Geneva 4, Switzerland
Author for correspondence (e-mail: beat.imhof@medecine.unige.ch)

Published on WWW 21 February 2000

SUMMARY

Leukocyte extravasation from the blood across the endothelium is vital for the functioning of the immune system. Our understanding of the early steps of this process has developed rapidly. However, it is still unclear how leukocytes undergo the final step, migrating through the junctions that mediate adhesion between adjacent endothelial cells, while preserving the barrier function of the endothelium. The first stage of transmigration – tethering and rolling – is mediated by interactions between selectins on the surface of leukocytes and glycosylated proteins such as GlyCAM-1 on the surface of endothelial cells. Stimulation of the leukocyte by chemokines then induces tight adhesion, which involves binding of activated leukocyte integrins to endothelial ICAM-1/VCAM-1 molecules. Passage of the leukocyte across the endothelium appears to require delocalization of certain endothelial cell molecules and proteolytic degradation of junctional complexes.

Key words: Leukocyte transendothelial migration, Vascular junctional molecule, Cell adhesion, Vascular permeability

LEUKOCYTES AND THE ENDOTHELIUM

The endothelium is extremely important in the regulation of immune responses: both innate immunity and adaptive immune responses depend upon the exit of white blood cells (leukocytes) from the blood and their migration across the endothelium to target tissues. Whereas innate immunity largely involves granulocytes (also called polymorphonuclear leukocytes, PMNs) and macrophages, adaptive immune responses require T and B lymphocytes. Together, the two systems provide an effective defence, so that we rarely become seriously ill, unless one or both of these systems fails.

For efficient protection of the body from infectious organisms, cells of both the innate and adaptive immune responses circulate as non-adherent cells in the blood and lymph, and migrate as adherent cells into tissues when necessary (for a review see Imhof and Dunon, 1995). Mature lymphocytes continuously recirculate between the blood and lymphoid tissues to seek out their cognate antigen (Ager, 1994; Pabst and Binns, 1989; Salmi and Jalkanen, 1997): this constitutes immune surveillance. Westermann and Pabst (1990, 1996) estimate that, in man, $\sim 5\times10^{11}$ lymphocytes leave the blood each day to enter various organs and then return to the blood. Other leukocytes, by contrast, migrate into tissues predominantly at sites of inflammation. To enter the lymphoid tissues the circulating leukocytes have first to adhere to and then to cross the endothelium. This process is not random, but involves active mechanisms of lymphocyte-endothelial-cell recognition, which are mediated by adhesion molecules (see below and Table 1).

The endothelium is made up of a continuous monolayer of cells linked by different types of adhesive structure; this forms a physical barrier to the passage of cells from blood to tissue. This barrier role must somehow be reconciled with a second, critical function of endothelial cells – that of serving as the point of entry for leukocytes into normal or inflamed tissue. This, perhaps paradoxical, role of the endothelial barrier is achieved by changes in its permeability. Such changes, therefore, constitute a key regulatory step in leukocyte recirculation, and any factors affecting this permeability should have a major effect on leukocyte transmigration. The importance of the endothelium in the control of leukocyte traffic is evident from its specialized morphology in areas of the body where the bulk of leukocyte transmigration takes place: the high endothelial venules.

HIGH ENDOTHELIAL VENULES

The process whereby leukocytes exit the bloodstream and migrate across the endothelium into the underlying tissue is generally referred to as extravasation. In lymphoid organs, lymphocyte extravasation occurs in the postcapillary venules (Gowans and Knight, 1964) at specialized postcapillary vascular sites called high endothelial venules (HEVs; Anderson and Anderson, 1976; Anderson et al., 1976). These, rather than non-specialized vessels, represent the major constitutive extravasation route in vivo (Lasky, 1992; Stoolman, 1989), and most recirculating lymphocytes selectively bind to the endothelium of HEVs, while ignoring

Table 1. Adhesion molecules involved in transendothelial migration

Cell Adhesion Molecule family	Expression	Ligand(s)	Adhesion step
SELECTINS			
L-selectin (MEL-14, CD62L)	Most non-activated leukocytes	sLe^x on GlyCAM, CD34 and MAdCAM-1	Tethering
P-selectin (CD62)	Activated platelets, inflammatory endothelium	PSGL-1	Tethering
E-selectin (CD62E)	Inflammatory endothelium	CLA, PSGL-1, ESL-1	Tethering
CARBOHYDRATE LIGANDS OF SELECTINS			
GlyCAM-1 (glycosylation dependent CAM)	High-endothelial venules	L-selectin	Tethering
CD34	Endothelium	L-selectin	Tethering
CLA (cutaneous lymphocyte antigen, carbohydrate structure on PSGL-1)	Activated lymphocytes	E-selectin	Tethering
MAdCAM-1 (mucosal addressin CAM)	Mucosal endothelium	L-selectin	Tethering
PSGL-1 (P-selectin glycoprotein ligand-1)	Myeloid, lymphoid and dendritic lineages	P-selectin, E-selectin	Tethering
ESL-1 (E-selectin ligand-1)	Myeloid cells	E-selectin	Tethering
INTEGRINS			
LFA-1 ($\alpha L\beta 2$)	Most leukocytes	ICAM-1,2,3	Tight adhesion
$\alpha 4\beta 1$ (VLA-4)	Lymphocytes	VCAM-1	Tethering and tight adhesion
$\alpha 4\beta 7$	Lymphocytes	VCAM-1 MAdCAM-1,	Tethering and tight adhesion
$\alpha M\beta 2$ (Mac-1),	Lymphocyte subsets	ICAM-1, fibrinogen, iC3b	Tight adhesion
$\alpha 9\beta 1$	Neutrophils, smooth muscle and endothelial cells	VCAM-1, tenascin-C	Tight adhesion
Ig SUPERFAMILY			
ICAM-1 (intercellular CAM-1)	Inflammatory endothelium, activated lymphocytes	LFA-1, $\alpha M\beta 2$	Tight adhesion
ICAM-2	Endothelium and resting lymphocytes	LFA-1	Tight adhesion
VCAM-1 (vascular CAM-1)	Inflammatory endothelium	$\alpha 4\beta 1$, $\alpha 4\beta 1$	Tight adhesion
MAdCAM-1	Mucosal endothelium	L-selectin, $\alpha 4\beta 7$	Tethering and tight adhesion

normal vascular endothelium (Hall and Morris, 1965; Mackay et al., 1990). This situation contrasts sharply with that at inflamed sites, where the damaged tissue triggers new adhesive properties in the adjacent endothelium. This results in local extravasation of leukocytes (including many lymphocytes), and among the earliest events in acute inflammation is the emigration of neutrophils from postcapillary venules and small veins (Grant, 1973; Hurley, 1983). In fact, neutrophils (phagocytic granulocytes) are the most numerous and important players in innate immunity: individuals suffering from hereditary deficiencies in neutrophil function fall victim to overwhelming bacterial infections that are fatal if left untreated.

In humans, HEVs are found in all secondary lymphoid organs (with the exception of the spleen). Endothelial cells of HEVs have a plump high-walled, cuboidal appearance that differs considerably from the flat morphology of other endothelial cells (Girard and Springer, 1995). They provide a striking example of control of endothelial differentiation by the local tissue environment. In fact, when peripheral lymph nodes are deprived of afferent lymph, HEVs convert to a flat-walled endothelial morphology and lose their ability to support lymphocyte traffic (Drayson and Ford, 1984; Hendriks and Eestermans, 1983). Additionally, HEV-like vessels are observed in chronically inflamed non-lymphoid tissues and are believed to support lymphocyte recruitment into these sites. This suggests that there is a direct link between the physical characteristics of HEVs and their ability to support leukocyte transmigration in these tissues. The cuboidal structure of HEVs results in a more irregular lining of the venules and thus an increased turbulence of the passing bloodstream. Consequently, leukocytes are more likely to collide with the endothelium and begin adhering and transmigrating (Girard and Springer, 1995; Kraal and Mebius, 1997). The marked HEV morphology is apparent in most species, including rodents and humans, although in certain species the height and cuboidal shape of the endothelial cell varies considerably. By contrast, sheep appear to lack HEVs (Kraal and Mebius, 1997), despite the fact that their functional capacity to sustain lymphocyte transmigration in lymphoid organs can be demonstrated.

LYMPHOCYTE HOMING

Many studies of transmigration have been concerned with ‘how lymphocytes know where to go’ (Salmi and Jalkanen, 1997), which is the first step in this process (a leukocyte needs to arrive at the right site before it can extravasate!). The migratory pathway is determined locally by the expression and activation of adhesion molecules, and is covered in detail elsewhere (Salmi and Jalkanen, 1997). The important point to consider is that the site at which a leukocyte extravasates is determined by the presence of an active receptor on the leukocyte and a corresponding ligand on the endothelial cell. These receptor-ligand pairs are members of a large family of adhesion molecules – surface receptors that determine the extent and specificity of leukocyte extravasation. Many adhesion molecules on endothelial cells are regulated by quantitative alterations in their surface expression (Carlos and Harlan, 1994; Pober and Cotran, 1990), being sparse or absent under normal physiological conditions, but readily induced or upregulated by inflammatory agonists (see below).

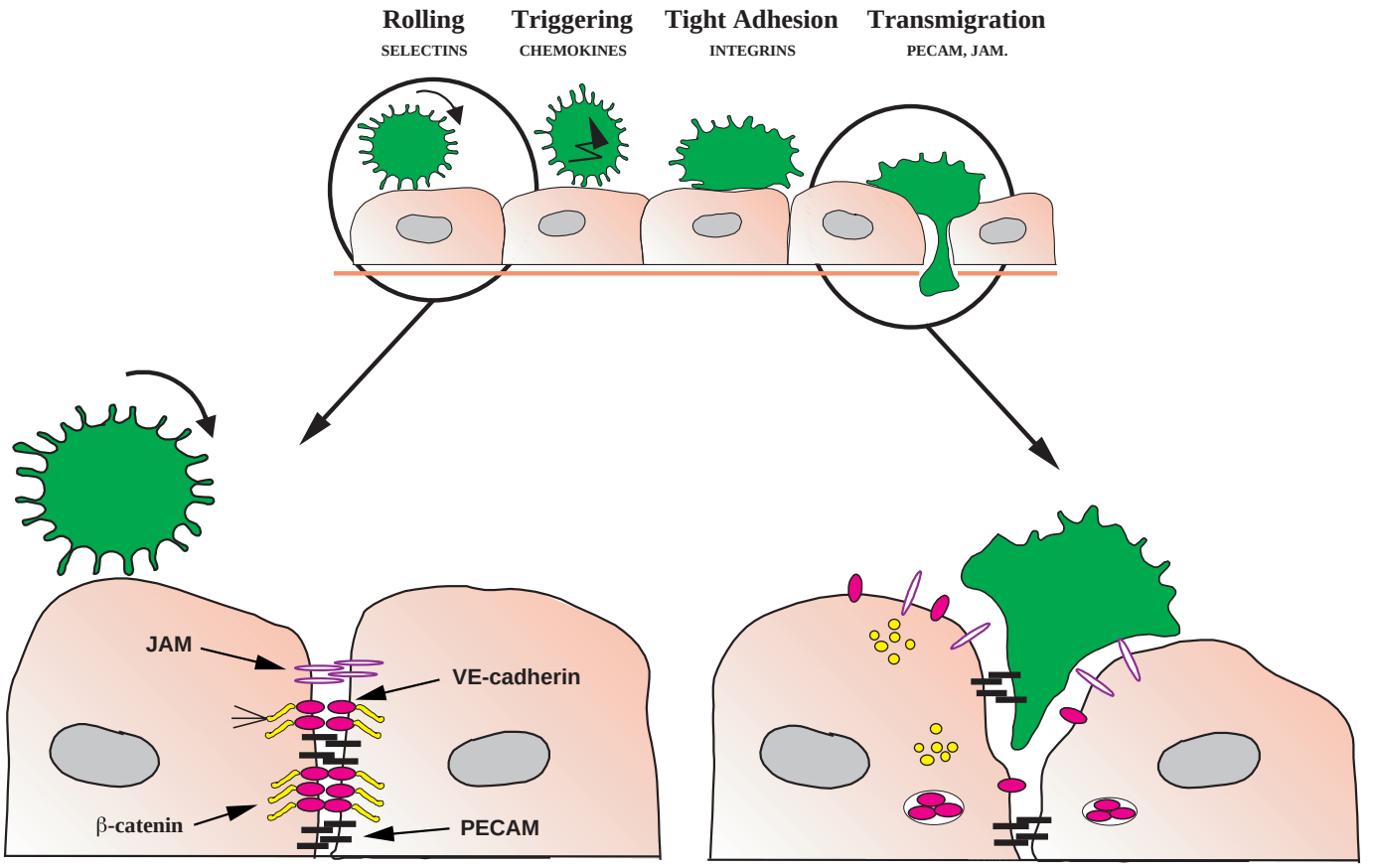

Fig. 1. Schematic representation of leukocyte transmigration and interendothelial contacts. In the 'resting state', the junction is closed, and this integrity is mediated by homotypic binding of VE-cadherin, PECAM-1 and JAM molecules. The VE-cadherin is linked to the cytoskeleton via β catenin, and this association is required for its adhesive activity. As it proceeds through the multi-step adhesion cascade, a leukocyte might trigger the dissociation of the VE-cadherin-β-catenin complex by degradation of the β-catenin and redistribution and/or internalization of the VE-cadherin. During transendothelial migration, the homophilic interactions (JAM-JAM and PECAM-1-PECAM-1) between opposing endothelial cells might be disrupted by the leukocyte, which would result in loosening of the junction. Engagement of PECAM-1 (or other endothelial molecules) might provide a scaffold to 'walk' the cell through the junction via haptotactic migration.

Most adhesion molecules involved in lymphocyte-endothelial-cell interactions are from one of five distinct molecular superfamilies: selectins, sialomucins, integrins, immunoglobulin-like and proteoglycans. Several comprehensive reviews on these molecules exist (Hynes, 1992; Imhof and Dunon, 1995; Salmi and Jalkanen, 1997; Shimizu et al., 1999) and, rather than give a detailed description here, we indicate the key molecules involved in each step of the transmigration process.

TRANSENDOTHELIAL MIGRATION

Leukocyte extravasation occurs both as part of a constitutive physiological process (during lymphocyte recirculation) and at sites of inflammation (in the case of granulocytes). In addition, monocytes constitutively exit the circulation at a low frequency to become tissue macrophages (van Furth, 1986). Many studies have demonstrated that leukocytes transmigrate by squeezing between adjacent endothelial cells and this is now the generally accepted view of transmigration, although it is not the exclusive pathway for transmigrating leukocytes. Indeed, early ultrastructural studies had suggested that leukocytes could pass through the body of an endothelial cell (Marchesi and Gowans, 1964) and, more recently, Feng et al. (1998) have demonstrated that neutrophils can emigrate from inflamed venules primarily by a transcytotic pathway in response to a chemotactic peptide. Serial electron microscopic sectioning showed clearly that the course taken by the transmigrating neutrophils did not involve interendothelial junctions (Feng et al., 1998). Nevertheless, the consensus of many experimental systems is that circulating leukocytes extravasate from venules through open interendothelial junctions. A multi-step model for this transmigration has been proposed (Carlos and Harlan, 1994; Springer, 1994): leukocyte rolling on endothelial cells; triggering by rapid activation of integrins via G-protein-linked receptors; tight adhesion to endothelial cells; diapedesis (in which the leukocyte 'crawls' through the junction between adjacent endothelial cells).

(i) Leukocyte rolling

The first step in leukocyte transmigration involves initial tethering, which is followed by reversible rolling on the endothelial surface. Given that this interaction involves a

leukocyte in flowing blood, it requires a rapid on-rate. A rapid off-rate is also necessary to prevent the indiscriminate and irreversible anchorage of leukocytes to vessel walls. Bjerknes et al. (1986) studied the dynamics of the attachment of lymphocytes to the endothelium of HEVs in murine Peyer's patches, using fluorescently labeled lymphocytes. They observed that labeled lymphocytes entered the HEV at high speed, collided with the endothelium, and came to a complete halt in less than 1/30 second after collision (Bjerknes et al., 1986). Most lymphocytes adhered to the HEV, but a majority of these quickly detached, most detachments occuring within the first few seconds.

Rolling is generally mediated by interaction between selectins and glycosylated ligands (Shimizu et al., 1999) and leads to a slowing down of the cells in the bloodstream, which enables them to detect signals from the endothelium. The selectin superfamily includes three adhesion molecules: L (leukocyte)-selectin, P (platelet)-selectin, and E (endothelial)-selectin. L-selectin is expressed only on leukocytes, whereas the other two selectins are found on endothelial cells (Lasky, 1995; Ley and Tedder, 1995; Rosen and Bertozzi, 1994; Vestweber, 1993). Models have been proposed that suggest that L-selectin and the ligands for P- or E-selectin are localized on the microvilli of leukocytes, where they are likely first to contact the vessel wall. This localization has been confirmed for L-selectin (Picker et al., 1991) and PSGL (a ligand for both E- and P-selectin; see below; Moore et al., 1995).

E-selectin is inducible, and its expression can be upregulated in as little as two hours (Wellicome et al., 1990). It mediates the binding of granulocytes and certain lymphocytes to activated endothelium. Interleukin 13 (IL-13) induces both E-selectin and P-selectin, and there is evidence for additive, synergistic or antagonistic effects of combinations of cytokines – for example, TNF-α and IFN-γ increase synthesis of E-selectin (Doukas and Pober, 1990; Leeuwenberg et al., 1990). In contrast to E-selectin, which is upregulated by de novo protein synthesis, P-selectin is sorted to specialized granules (Weibel-Palade bodies), and translocates to the cell surface within minutes of stimulation by thrombin, histamine, calcium ionophores and complement components (Hattori et al., 1989; McEver et al., 1989). E- and P-selectin share PSGL-1 as a ligand (see Table 1).

L-selectin is present on all naive mature lymphocytes, whereas memory cells show bimodal expression: a subpopulation expresses higher levels than naive cells, whereas the rest do not express the protein (Picker et al., 1993). L-selectin is also present on neutrophils and monocytes. Activation of lymphocytes can lead to a transient increase in L-selectin activity before the receptor is shed from the lymphocyte surface. The expression of L-selectin is also controlled by several cytokines (Evans et al., 1993; Griffin et al., 1990). The ligands for L-selectin include glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1), CD34, podocalyxin and MAdCAM-1 (Rosen, 1999). MAdCAM-1 plays a role principally in the homing of naive lymphocytes to gut-associated lymphoid tissues (Butcher et al., 1999; Nakache et al., 1989; Streeter et al., 1988), interacting with the $\alpha_4\beta_7$ integrin. However, in some tissues it displays a particular carbohydrate moiety, sLe^x, that serves as a ligand for L-selectin (Bargatze et al., 1995; Berg et al., 1993). Since these ligands are constitutively expressed as L-selectin-binding glycoforms by HEVs, the involvement of L-selectin in inflammatory processes requires the inducibility of additional endothelial ligands the identity of which is not yet clear. Candidates for such molecules include E-selectin (for neutrophils but not for lymphocytes; Picker et al., 1991) and cutaneous lymphocyte antigen (CLA; Tu et al., 1999).

Until recently, the rolling phase was thought to be mediated solely by the selectins. However, the integrin VLA-4 can support tethering and rolling in flow on VCAM-1, an endothelial integrin ligand that belongs to the immunoglobulin superfamily (Alon et al., 1995).

(ii) Activation of integrins

The second step in the process of transmigration involves stimulation of the leukocyte by chemokines. Chemokines play crucial roles in immune and inflammatory reactions (Baggiolini, 1998; Hedrick and Zlotnik, 1996), and ~50 of these molecules have been identified in humans. Many chemokines are produced upon cell activation – for example, endothelial cells exposed to bacterial products of primary inflammatory cytokines (lipopolysaccharide, tumour necrosis factor-α and interleukin-1) produce monocyte chemotactic proteins (MCPs), RANTES, fractalkine and macrophage inflammatory proteins (MIPs; Baggiolini et al., 1994; Ben-Baruch et al., 1995; Mantovani et al., 1997, 1998). Some chemokines (e.g. TARC, ELC, SLC, LARC and DC-CK1), however, seem to fulfill housekeeping functions and are constitutively expressed (Baggiolini, 1998). All of these (with the exception of LARC) are constitutively expressed at high levels in the thymus, lymph nodes and certain lymphoid tissues.

It has been demonstrated for certain chemokines that they associate, through a heparin-binding domain, with proteoglycans on the surface of endothelial cells, which then present the chemokine at the luminal surface of the endothelium (Tanaka et al., 1993). The effects of chemokines on leukocytes are mediated by G-protein-coupled receptors, and leukocyte responses to these molecules depend on the presence of the relevant receptor, which determines the spectrum of action of the chemokines. In addition, the use of distinct chemokines at specific anatomical sites contributes to the specificity of leukocyte recruitment. Chemokines have redundant effects on target cells and, usually, a given leukocyte population has receptors for and responds to several different chemokines (Mantovani, 1999).

The eponymous function of chemokines is chemotaxis for leukocytes, and chemokines are seen as the family of proteins that regulates leukocyte migration. This migration is controlled at two critical points: firstly, activation of the leukocyte, which leads to its arrest and tight adhesion onto the endothelial cell surface; and, secondly, the migration of the leukocyte through the tissues to target microenvironments (after it has crossed the endothelium). Chemokines are critical for both of these steps, but here we restrict our discussion of their action to the first step.

Several chemokines induce adhesion of human lymphocytes to endothelial cells or endothelial ligands – including macrophage inflammatory protein-1α (MIP-1α; Lloyd et al., 1996; Taub et al., 1993a), MIP-1β (Lloyd et al., 1996; Tanaka et al., 1993; Taub et al., 1993a) interferon-α-inducible protein 10 (IP10; Lloyd et al., 1996; Taub et al.,

1993b) and RANTES (Lloyd et al., 1996; Taub et al., 1993a,b). However, Campbell et al. (1998) have recently suggested that these chemokines are relatively slow acting and often require prior cellular stimulation; thus they might not trigger rapid adhesion of normal leukocytes to the endothelium effectively (but rather mimic chemokine stimulation of activated immunoblasts within the tissues). Recently, Campbell et al. (1998) have identified four chemokines (stromal cell-derived factor-1α (SDF-1α), SDF-1β, macrophage inflammatory protein-3β (MIP-3β), and 6-C-kine) that can trigger almost immediate integrin-dependent arrest of lymphocytes under physiological shear. The rapidity of the response is critical because leukocytes rolling through a site of inflammation must be stopped before exiting the area relevant to the adhesion-triggering stimulus. These newly described chemokines might therefore be more relevant to the rapid adhesion of normal lymphocytes observed during physiological lymphocyte-endothelial-cell interactions in vivo (Carr et al., 1996).

The stimulation of a leukocyte through chemokine receptors results in an increase in integrin activity and thus increased adhesion of the leukocyte to members of the immunoglobulin superfamily present on the endothelial cell surface. This brings about stable arrest of the leukocyte on the endothelium.

Although chemokine stimulation is the generally accepted mechanism that leads to integrin activation, a second mechanism that involves selectin-mediated signaling has been described. Neutrophils adherent to E-selectin on IL-1-prestimulated endothelial cells have been reported to increase the adhesive activity and expression of the integrin $\alpha_M\beta_2$ (Mac-1; Kuijpers et al., 1991; Lo et al., 1991). In addition, Simon et al. (1995) showed that cross-linking L-selectin induced significant adhesion and transmigration of neutrophils across HUVEC monolayers. They found that cross-linking L-selectin with antibody increased the surface expression of Mac-1, as well as increased expression of the activated form of the β_2 integrin (Dransfield and Hogg, 1989). Furthermore, cross-linking L-selectin increased Mac-1-dependent adhesion and transmigration of neutrophils on LPS-stimulated human umbilical vein endothelial cell (HUVEC) monolayers. This adherence and transmigration could be blocked by antibody to either the β_2 integrin or to the endothelial ligand ICAM-1 (Simon et al., 1995). In a similar study, Hwang et al. (1996) showed that ligation of L-selectin via either GlyCAM-1 or antibody cross-linking enhances β_2 integrin function in naive peripheral lymphocytes and, consequently, increases adhesion of the cells to melanoma cells transfected with ICAM-1.

(iii) Tight adhesion

Integrins are heterodimers formed by a combination of one of 17 α and 8 β chains in humans (Shimizu et al., 1999). The integrins α4β1(VLA-4), αLβ2 (LFA-1), Mac-1 and α4β7 have major functions in leukocyte-endothelial cell interactions (Hu et al., 1993; Larson and Springer, 1990; Lobb and Hemler, 1994; Stewart et al., 1995). LFA-1 is almost ubiquitously expressed on normal lymphocytes, and the highest levels are seen on activated cells. The regulation of integrin activity can be achieved in several ways: by a change in integrin affinity (Ginsberg et al., 1992), by association of the integrin with the cytoskeleton (Kucik et al., 1996) or by clustering (van Kooyk et al., 1994). For example, activation of LFA-1 is accompanied by a conformational change that exposes cryptic activation-dependent epitopes (Dransfield and Hogg, 1989) and increases binding affinity (Dustin and Springer, 1989; van Kooyk et al., 1989).

The ligands for these integrins are members of the Ig superfamily: ICAM-1 and ICAM-2 bind to LFA-1, and VCAM-1 binds to VLA-4. ICAM-1 and VCAM-1 are upregulated on inflamed endothelium by IL-1, TNF-α and LPS (Dustin et al., 1986; Wellicome et al., 1990). In addition, IL-4 and IL-13 induce synthesis of VCAM-1 (Masinovsky et al., 1990; Sironi et al., 1994), IFN-γ upregulates ICAM-1 (Carlos et al., 1990), whereas TNF-α and IL-4 augment VCAM-1 but inhibit ICAM-1 expression (Masinovsky et al., 1990; Thornhill and Haskard, 1990). Mechano-stimulation also regulates the expression of certain adhesion molecules on endothelial cells, and ICAM-1 is induced by a constant shear stress in human umbilical vein endothelial cell (HUVEC) monolayers (Nagel et al., 1994).

(iv) Diapedesis

Diapedesis through the vessel wall is the final step in the transmigration process. Although an understanding of the early events (i.e. steps 1-3) has proceeded rapidly, it is still far from clear how leukocytes ‘crawl’ through the endothelial wall lining the blood vessels. The transmigrating leukocyte must somehow induce the endothelium to open the contact between adjacent cells in order to allow its passage between them. In the remainder of this article, we give an overview of the key molecules currently known to be present at the junction between endothelial cells and discuss their roles in the final step of transendothelial migration.

INTERCELLULAR JUNCTIONS BETWEEN ENDOTHELIAL CELLS

Although trancytosis has been convincingly demonstrated (Feng et al., 1998), the most often-described route for a leukocyte across the endothelium is through the gap between pairs of adjacent cells (bicellular migration). This is a daunting prospect because endothelial cells possess a number of junctions that maintain the integrity of the endothelium and regulate vascular permeability. Interestingly, Burns et al. (1997) have demonstrated that neutrophil transendothelial migration occurs preferentially at tricellular corners, where the borders of three endothelial cells intersect. Immunofluorescence studies confirmed that both tight junctions and adherens junctions are discontinuous at tricellular corners. We discuss these findings in more detail below.

At least three types of endothelial cell junctions have been described: gap junctions, tight junctions (TJs) and adherens junctions (AJs; Anderson et al., 1993; Beyer, 1993; Gumbiner, 1993; Rubin, 1992; Schmelz and Franke, 1993). These junctions were defined morphologically and are formed by a complex network of transmembrane proteins that are specific to each type of junction (Dejana et al., 1995).

Gap junctions

Gap junctions are clusters of transmembrane hydrophilic

channels that allow direct exchange of ions and small molecules between adjacent cells (Beyer, 1993). In vivo, the frequency of gap junctions correlates with that of tight junctions, and the two are usually intercalated.

Tight junctions

TJs (also called zonula occludens) are the most apical junctions (closest to the luminal surface) and form very close contacts between adjacent cells. They appear as a series of discrete sites of apparent membrane fusion, involving the outer leaflet of the plasma membranes of adjacent cells. Three types of transmembrane protein, occludin (Furuse et al., 1993), claudins (Furuse et al., 1998) and junctional adhesion molecule (Martin-Padura et al., 1998), co-localize with TJs. Inside the cells, several cytoskeletal signaling molecules are concentrated in the TJ area, including zonula occludins 1 (ZO-1) and cingulin (Gumbiner, 1996). Interestingly, the number of tight junctions in endothelial cells varies inversely in relation to the requirement for permeability control (Simionescu and Simionescu, 1991) and, whereas the endothelium of large arteries has a high frequency of TJs, the endothelium of the post-capillary venules within lymphoid tissues (where the EC are of the HEV phenotype) is relatively devoid of TJs. The latter finding is consistent with the role of the HEVs in supporting the passage of extravasating leukocytes in the lymphoid organs. The evidence for the absence of TJs is based on a morphological definition however, and some of the proteins participating in epithelial cell TJs might be present in endothelial cells of HEVs, including ZO-1 (our unpublished observations) and JAM (Malergue et al., 1998). Outside lymphoid tissues, post-capillary venules exhibit organized tight junctions (Bowman et al., 1991).

Adherens junctions

With respect to leukocyte extravasation, the adherens junctions (AJs) are of particular interest because they appear to be the main complex regulating macromolecular permeability in microvascular endothelium. Adherens junctions (also called zonula adherens) are cellular membrane contacts formed by cadherins. These transmembrane glycoproteins physically attach the cell membrane to an intracellular undercoat network of cytoplasmic proteins and actin microfilaments (Geiger and Ayalon, 1992; Tsukita et al., 1992). Cadherins are single-chain proteins comprising a highly conserved cytoplasmic region and an extracellular domain that contains calcium-binding motifs. They promote homophilic, calcium-dependent cell-cell adhesion (Kemler, 1993; Takeichi, 1991). To exhibit functional adhesive activity, cadherins interact with the actin cytoskeleton through their cytoplasmic tails, the association being mediated by the intracellular catenins, α-catenin, β-catenin and plakoglobin (γ-catenin; Kemler, 1993; Tsukita et al., 1992). In addition to neuronal cadherins, the endothelium expresses a specific cadherin, VE-cadherin, which localizes at intercellular junctions in all endothelia (Ayalon et al., 1994; Lampugnani et al., 1992) and which, in a similar way to the other cadherins, forms complexes with the catenins (Lampugnani et al., 1995). VE-cadherin is a target of agents that increase vascular permeability, such as vascular endothelial growth factor, VEGF, (a potent inducer of new blood vessels and vascular permeability in vivo; Esser et al., 1998), histamine and thrombin (Rabiet et al., 1994, 1996).

TRANSMIGRATION AND VASCULAR PERMEABILITY

The correlation between leukocyte transmigration and changes in vascular permeability is controversial. It has been demonstrated that migration of leukocytes towards endothelial cell-cell junctions, and subsequent transmigration, is frequently accompanied by an increase in vascular permeability (Del Maschio et al., 1996; Larsen et al., 1980; Tinsley et al., 1999). However, Huang et al. (1988) showed that migration of PMNs through HUVEC monolayers in response to chemoattractants did not induce any change in electrical resistance or albumin permeability of these monolayers. Similarly, in a subsequent study, Huang et al. (1993) reported that migration of PMNs across HUVEC monolayers (at a PMN:endothelial-cell ratio of 10:1) induced no change in transendothelial electrical resistance. However, in the same study, the authors found that at larger numbers of PMNs (25:1 and 50:1) transmigration was accompanied by large decrease in electrical resistance. In addition, the adhesion of chemokine-stimulated PMNs to HUVEC monolayers (in the absence of transmigration) induced a large decrease in HUVEC electrical resistance (Huang et al., 1993). The authors conclude that the PMN:endothelial-cell ratio is a determinant of changes in permeability: at lower ratios the transmigrating PMNs ‘plugs’ the gap between the endothelial cells, whereas at higher ratios, the presence of larger numbers of PMNs in the intercellular spaces results in increased permeability to ions. Similarly, where the PMNs adhere but do not transmigrate, the gaps induced as a result of signals from the activated PMNs are not ‘plugged’; hence, the permeability of the monolayer to ions is increased (Huang et al., 1993). (Interestingly, in the studies by Del Maschio et al. and Tinsley et al., the authors looked at the effects of PMN adhesion rather than transmigration, and so the increased permeability they observe would be consistent with these conclusions.)

It is reasonable to envisage that, in a physiological context where activated leukocytes are both adhering to and transmigrating the endothelium, net changes in vascular permeability might be very small or negligible (since even large increases induced by adhesion of leukocytes could be abrogated if the leukocytes are able to fill in the gaps as they transmigrate). Indeed, large changes in vascular permeability would be undesirable, since they would compromise the barrier function of the endothelium.

CADHERINS AND VASCULAR PERMEABILITY

Several studies have demonstrated that VE-cadherin could be an important determinant of vascular permeability (Corada et al., 1999; Hordijk et al., 1999). Hordijk and colleagues showed, in HUVECs, that VE-cadherin preferentially co-localizes with actin stress fibers and that antibody blocking of VE-cadherin led to a marked reorganization of the actin cytoskeleton, increased monolayer permeability and enhanced neutrophil transmigration. These effects of antibody treatment were accompanied by a change in the distribution of VE-cadherin, and immunostaining showed that VE-cadherin was no longer localized to sites of cell-cell contact but instead diffusely distributed (although not internalized). In addition, Corada et al. (1999) showed that, in murine endothelial cells, an antibody

directed against VE-cadherin induced a diffuse redistribution of VE-cadherin on the cell surface away from intercellular junctions. In vivo administration of this antibody resulted in increased vascular permeability in heart and lungs.

Esser et al. (1998) propose that tyrosine phosphorylation of VE-cadherin regulates vascular permeability. In an in vitro model, VEGF stimulated the migration of endothelial cells and induced an increase in the paracellular permeability of HUVECs. These effects correlated with increased phosphotyrosine labeling at cell-cell contacts, and biochemical analyses revealed that 15 minutes to one hour after VEGF stimulation, VE-cadherin, β-catenin, plakoglobin and p120 were maximally phosphorylated on tyrosine (Esser et al., 1998). What the role of this phosphorylation might be is not yet clear. Interestingly, the authors observed a striking qualitative (although no significant quantitative) change in VE-cadherin staining. 10 minutes to one hour after VEGF addition (when VEGF-induced cadherin and catenin phosphorylation was maximal), the VE-cadherin staining at cell-cell contacts redistributed in a zig-zag pattern. These findings are consistent with earlier observations that link tyrosine phosphorylation of junctional proteins with loss of integrity of intercellular adhesions (Behrens et al., 1993; Matsuyoshi et al., 1992).

Because VE-cadherin could play a key role in regulating vascular permeability, a pertinent question is that of whether a transmigrating leukocyte can affect VE-cadherin distribution, perhaps following activation by chemokines. This would 'target' the endothelial opening to precisely where a gap was required (see below).

THE ROLE OF LEUKOCYTES IN THE REGULATION OF VASCULAR PERMEABILITY

Del Maschio and co-workers (1996) demonstrated that adhesion of PMNs to endothelial cells disrupts the VE-cadherin-catenin complex. These molecules are lost from cell-cell contacts, and a large amount of β-catenin and plakoglobin disappears from VE-cadherin immunoprecipitates and total cell extracts. This effect is rapid, being maximal within 5 minutes, and was seen only when PMNs adhered to TNF-treated (but not resting) endothelial cell monolayers. The loss of VE-cadherin staining at the cell surface was partly due to its internalization, although most of the molecules probably simply diffuse on the cell surface. The authors conclude that the disruption of the VE-cadherin-catenin complex is not mediated by the extracellular release of soluble reactive components, such as proteases or oxygen-reactive metabolites produced by the PMNs, but instead depends on adhesion of PMNs to the endothelial cell monolayer.

The disorganization of the VE-cadherin complex induced by PMN adhesion was accompanied by an increase in endothelial permeability. One possibility Del Maschio et al. considered is that, through their adhesion, PMNs induce a series of intracellular responses in the endothelial cells that results in the detachment of catenins from VE-cadherin. Previous reports had demonstrated that, during adhesion of PMNs and NK cells, the cytosolic calcium level in endothelial cells increases (Huang et al., 1993; Pfau et al., 1995). Consequently, Del Maschio and co-authors suggest that this increase in cytosolic calcium activates calpain, a calcium-activated protease, which in turn might be responsible for dissociation of the VE-cadherin-catenin complex and catenin lysis. Allport and co-workers (1997) first suggested that an endothelial protease is involved in disruption of the VE-cadherin complex: they showed that, in a HUVEC model, neutrophil adhesion dramatically disrupts the VE-cadherin complex and induces partial degradation of members of the complex. Neutrophil membranes were sufficient to obtain this effect, which excludes the possibility that the degradation is mediated by neutrophil granule proteolytic enzymes. The authors concluded that contact between the leukocyte and the endothelial layer was required for endothelial-mediated VE-cadherin disruption, but that the endothelial proteasome system was not involved (although inhibitors of the proteasome prevented >60% of neutrophil transmigration, which suggests that signal transduction events initiated by endothelium-leukocyte interactions require the proteasome pathway).

The above findings support an attractive model for leukocyte-induced endothelial regulation of vascular permeability. However, more-recent studies have indicated that the adhesion-dependent disappearance of endothelial catenins might not, in fact, be mediated by a specific leukocyte-to-endothelium signaling event, but might rather be due to the activity of a neutrophil protease released during the experimental procedure; thus the observations might be a consequence of a nonspecific proteolytic event (Moll et al., 1998). Experiments by Carden and co-workers (1998) support this hypothesis. They demonstrated that neutrophil elastase (a serine protease released upon neutrophil activation) degrades endothelial cadherins. The amount of VE-cadherin in HUVECs was significantly reduced by exposure to activated neutrophils, and this degradation of VE-cadherin was attenuated by administration of a specific elastase inhibitor. At present, therefore, available data suggest that both neutrophil-derived and endothelium-derived proteases cause partial degradation of the VE-cadherin complex. The 'specificity' of neutrophil proteases could be controlled by the requirement for local activation of the neutrophil at the surface of the endothelial cell, as well as by the short-range action of the protease (which is due to the presence of protease inhibitors in blood plasma). Interestingly, Cepinskas et al. (1999) recently demonstrated that platelet activating factor (PAF)-activated PMNs mobilize their endogenous elastase to the cell surface and that transmigration of these cells across HUVEC monolayers is inhibited (66%) by a mAb against elastase. Furthermore, using laser scanning confocal microscopy, the authors demonstrate that these PAF-activated PMNs redistribute their membrane-bound elastase to the migrating front – i.e. to sites of interaction with the endothelium (the pseudopodia). Such a mechanism would 'localize' the protease to the endothelial cell being traversed.

The idea that the adhesion (and activation) of leukocytes affects the VE-cadherin-catenin complex remains an intriguing hypothesis. In a recent study: Tinsley et al. (1999) propose that neutrophils regulate endothelial barrier function through a process involving conformational changes in β-catenin and VE-cadherin. They show that treatment of cultured bovine coronary endothelial monolayers with activated neutrophils results in an increase in permeability and large-scale intercellular gap formation. There is loss of β-catenin staining in areas where individual cells have lost contact with

neighbouring cells. At the same time, VE-cadherin staining in cells exposed to activated neutrophils changes from a uniform distribution along the membrane to a diffuse pattern, in which little if any VE-cadherin remains at the cell periphery. Actin stress fiber formation increased in treated cells, and β-catenin and VE-cadherin from neutrophil-treated cells showed a significant increase in tyrosine phosphorylation. These observations provide evidence for specific neutrophil-mediated changes in adherens junctions, intercellular gap formation and hyperpermeability.

TYROSINE PHOSPHORYLATION AND VASCULAR PERMEABILITY

The correlation between tyrosine phosphorylation of the VE-cadherin complex and changes in vascular permeability is reminiscent of earlier findings suggesting that tyrosine phosphorylation is involved in the barrier function of endothelial cells. For example, phosphorylation of myosin light chain can induce actin polymerization, endothelial cell centripetal retraction and a subsequent increase in endothelial permeability (Goeckeler and Wysolmerski, 1995; Moy et al., 1993, 1996; Sheldon et al., 1993). In addition, tyrosine phosphorylation of two focal-adhesion-associated proteins, paxillin and $pp125^{FAK}$, in coronary venular endothelial cells correlates with increased microvascular endothelial permeability, and inhibition of tyrosine phosphorylation prevented the hyperpermeability response of venules to inflammatory agonists (Yuan et al., 1998).

Stimulated neutrophils induce myosin light chain phosphorylation via activation of myosin light chain kinase (MLCK), as well as isometric tension in endothelial cell monolayers (Hixenbaugh et al., 1997), whereas unstimulated PMNs have no effect on either parameter. The generation of tension preceded migration of the PMNs across the endothelium, and thus the authors suggest that chemoattractant-stimulated PMNs induce endothelial cells to open their intercellular junctions through phosphorylation of myosin regulatory light chains. This leads to increased endothelial cell monolayer isometric tension and thereby a loosening of junctional contacts between endothelial cells. Saito et al. (1998) obtained similar results in a study in which they additionally show that pretreatment of HUVECs with an MLCK-specific inhibitor diminished migration of neutrophils across the HUVEC monolayer.

THE ROLE OF TJS IN TRANSENDOTHELIAL MIGRATION

The contribution made by tight junctions to the regulation of leukocyte diapedesis has been largely ignored by many investigators. This omission is unjustified, and is mainly due to experimental limitations: HUVEC monolayers are the most commonly used endothelial cells for in vitro studies of leukocyte transendothelial migration and they rarely form TJs in vitro, except under particular conditions (Burns et al., 1997, 2000). Since TJs are situated closest to the luminal surface, they are strategically placed to regulate leukocyte transendothelial migration. Tight junctions have discontinuities at points of contacts between adjacent vascular endothelial cells, as well as at tricellular corners (Simionescu et al., 1976; Walker et al., 1994; Yee and Revel, 1975). A number of earlier reports had shown that brain astrocytes and their secreted products can induce and maintain TJs in cultured microvascular and aortic endothelia (Shivers et al., 1988; Wolburg et al., 1994). In addition, Tio et al. (1990) published data showing that astrocytes can also induce tight-junction formation in primary HUVEC cultures. Burns et al. (1997) used a similar approach to detemine whether TJs play a role in regulating neutrophil transendothelial migration. The addition of endothelial cell growth supplement to the medium was necessary and sufficient for tight junction expression. However, transcellular electrical resistance and tight junction frequency were enhanced by culturing of monolayers of HUVECs in astrocyte-conditioned medium. Interestingly, transmigration of neutrophils in IL-1-treated HUVEC monolayers was similar in the presence or absence of TJs (Burns et al., 1997). Finally, immunofluorescence studies confirmed that both TJs and AJs are discontinuous at tricellular corners and that tricellular corners are the preferred site for neutrophil transmigration. In a recent study, Burns et al. (2000) demonstrate that migration of PMNs across HUVEC monolayers does not result in widespread proteolytic loss of TJ proteins (ZO-1, ZO-2 and occludin) from endothelial borders. Ultrastructurally, TJs appear intact during PMN transendothelial migration. Furthermore, transendothelial electrical resistance is unaffected by PMN adhesion and migration, which demonstrates that preservation of the endothelial barrier property during PMN migration is associated with maintenance of the TJ complex. Both studies by Burns suggest that the majority of PMNs migrate around TJs by crossing at tricellular corners rather than passing through and disrupting the junctions that lie between pairs of endothelial cells. Thus the transmigrating leukocyte might make a ‘choice’: either to induce disruption of the junctional complexes, as is suggested for AJs, or to ‘bypass’ the junctions and cross the endothelium at regions where the junctions are discontinuous. This would have the dual advantage of requiring perhaps less ‘effort’ from the transmigrating leukocyte while maintaining the integrity of the endothelium.

OTHER PROTEINS AT CELL–CELL CONTACT SITES

In addition to the cadherins, which localize to AJs, several other molecules are also concentrated at endothelial cell junctions. Of these, PECAM-1 (CD31) and JAM have been suggested to play an active role in the transendothelial migration of leukocytes.

PECAM-1

PECAM-1 is a member of the Ig superfamily and is a single-chain transmembrane glycoprotein that contains six Ig-like extracellular domains, a transmembrane portion and a cytoplasmic tail. PECAM-1 is expressed diffusely on the surfaces of platelets and most leukocytes (DeLisser et al., 1994; Watt et al., 1995). It is also present on endothelial cells, where it is concentrated at the intercellular junctions but not associated with either AJs or TJs (Ayalon et al., 1994). PECAM-1 can support cell-cell adhesion through either

homophilic (Newton et al., 1997; Sun et al., 1996) or heterophilic interaction (Muller et al., 1992), and several laboratories have reported that anti-PECAM-1 mAbs cause activation of β1 (Leavesley, 1994; Tanaka et al., 1992), β2 (Berman et al., 1996; Piali et al., 1993) and β3 integrins (Varon et al., 1998; Chiba et al., 1999). Proposed heterophilic ligands include the integrin $\alpha_V\beta_3$ (Buckley et al., 1996; Piali et al., 1995) and other uncharacterized molecules on activated T cells (Prager et al., 1996). Although PECAM-1 has been demonstrated to play a role in the initiation of endothelial cell contact, endothelial cell tube formation and in vivo angiogenesis (Albelda et al., 1990; DeLisser et al., 1997), this role is apparently functionally redundant: PECAM-1-negative mice are viable and undergo normal vascular development (Duncan et al., 1999). PECAM-1 has also been implicated as a critical mediator of transendothelial migration (Muller et al., 1993), although the absence of PECAM-1 in knockout mice does not affect the number of monocytes and lymphocytes undergoing transendothelial migration in response to thioglycolate, IL-1β and formalin-inactivated *S. aureus* (Duncan et al., 1999). To date, the only reported defect in PECAM-1-deficient mice is an accumulation of polymorphonuclear cells at the basement membrane of postcapillary venules in the mesentery of animals after intraperitoneal injection of IL-1β (Duncan et al., 1999). PECAM-1 might therefore play a role in the migration of PMNs across the transbasement membrane.

Recently, Matsumura and co-workers (1997) demonstrated that β-catenin and PECAM-1 interact. These studies have since been extended to show that this association positively correlates with the level of β-catenin phosphorylation (Ilan et al., 1999). In addition to its role in adherens junction formation and function, β-catenin can act as a signaling molecule, bind transcription factors and translocate to the nucleus. One way to regulate catenin-based signaling is by controlling the cytoplasmic levels of catenins. Indeed, β catenin is tightly controlled by incorporation into junctional complexes and by complexing with APC, GSK-3β and axin (Brown and Moon, 1998; Ikeda et al., 1998; Sakanaka et al., 1998). Ilan and co-workers (1999) suggest that PECAM-1 plays a role in control of the cytoplasmic levels of β-catenin by recruiting it to the plasma membrane and by bringing SHP-2 into close proximity, which results in dephosphorylation of β-catenin. The physiological relevance of the PECAM-1-β-catenin/SHP-2 interaction, and the role of tyrosine phosphorylation of β-catenin in endothelial cell adhesion and permeability, is, as yet, undetermined. It is tempting to speculate that the recruitment of phosphatases by PECAM-1 to the endothelial cell-cell junction is linked to its suggested role in transendothelial migration – perhaps via the dephosphorylation of junctional molecules whose function in regulating the closure of the junction depends upon their levels of tyrosine phosphorylation. However, such target molecules remain to be elucidated, and the role of PECAM-1 in transendothelial migration is far from clear.

JAM

JAM (junctional adhesion molecule), like PECAM-1, is a member of the Ig superfamily that was recently identified and cloned (Martin-Padura et al., 1998). JAM interacts homotypically in confluent monolayers of transfected CHO cells, and confocal and immunoelectron microscopy have shown that it co-distributes with TJ components. An antibody directed against JAM (BV11) inhibited spontaneous and chemokine-induced monocyte transmigration through an endothelial cell monolayer in vitro and monocyte infiltration in a model of skin inflammation in mice (Martin-Padura et al., 1998). The role of JAM in transendothelial migration is, to date, unknown. Its presence in the TJ, along with the effect of antibody treatment on transendothelial migration (Martin-Padura et al., 1998), is intriguing and suggests that proteins associated with TJs play a role in the regulation of leukocyte extravasation across endothelia that possess such junctions.

Martin-Padura et al. (1998) suggest that JAM binds to monocytes and directs their migration through the intercellular cleft. As the anti-JAM antibody did not recognize the monocytes, it seems unlikely that that the effect of the antibody on transendothelial migration was simply an inhibition of homotypic binding (especially given that addition of the antibody did not change endothelial cell or JAM-CHO transfectant paracellular permeability). Interestingly, JAM is concentrated at the apical regions of intercellular junctions (where TJs are located), where it might be readily engaged by a cell that has already adhered to the surface of an endothelial cell. PECAM-1 is located in a less apical domain of the inter-endothelial-cell cleft (Ayalon et al., 1994); these two proteins might therefore collaborate in 'guiding' the cell through the inter-endothelial-cell junction. Ozaki and co-workers (1999) have recently cloned human and bovine homologues of JAM and demonstrated regulation of JAM localization in HUVECs. This study demonstrates that the combined treatment of HUVECs with TNF-α plus IFN-γ caused a disappearance of JAM from intercellular junctions and its re-distribution on the cell surface, (the total amount of JAM was not reduced). The authors suggest that this redistribution of JAM partly accounts for the reduction in leukocyte transendothelial migration that had been observed after combined action of TNF-α and IFN-γ on human endothelial cells (Rival et al., 1996). Experiments carried out by Rival and co-workers had correlated the decrease in transmigration with a decrease in the amount of PECAM-1 at intercellular junctions, but perhaps a redistribution of JAM also played a role (although note that the decrease in PECAM-1 levels at intercellular junctions was accompanied by increased expression of ICAM-1 and, perhaps more significantly, by increased adhesion on the surface of the endothelial cell). If JAM does indeed play a role in transendothelial migration, then its effects appear to be restricted to monocytes (Martin-Padura et al., 1998).

CONCLUDING REMARKS

In 1997 Salmi and Jalkanen asked: 'How do lymphocytes know where to go?' Our question is 'How do leukocytes cross the barrier when they arrive?' There is overwhelming evidence for the role of the VE-cadherin complex in regulating vascular permeability, and the complex might in turn be regulated by adhering leukocytes. Diapedesis almost certainly involves more than one step, given that each molecule present at the interendothelial junction represents a potential barrier that the transmigrating leukocyte must pass. It seems probable that transmigration involves delocalization of some endothelial

molecules and proteolytic degradation of certain members of junctional complexes. In addition, the transmigrating leukocyte probably actively engages other endothelial molecules, using them as a 'scaffold' for haptotactic migration across the across the vessel wall (Bianchi et al., 1997). This would make biological sense: the leukocyte would act as a 'plug' as it proceeds through the gap in the endothelium (by the physical engagement of junctional molecules), with minimal leakage of solutes at the endothelial barrier.

The key to the door is not yet in our hands, although the shape of the lock it must fit becomes increasingly better defined.

The authors thank Drs Christiane Ody and Guido Wiedle for helpful comments and critical review of the manuscript.

REFERENCES

Ager, A. (1994). Lymphocyte recirculation and homing: roles of adhesion molecules and chemoattractants. *Trends Cell Biol.* **4**, 326-333.

Albelda, S. M., Oliver, P. D., Romer, L. H. and Buck, C. A. (1990). EndoCAM: a novel endothelial cell-cell adhesion molecule. *J. Cell Biol.* **110**, 1227-1237.

Allport, J. R., Ding, H., Collins, T., Gerritsen, M. E. and Luscinskas, F. W. (1997). Endothelial-dependent mechanisms regulate leukocyte transmigration: a process involving the proteasome and disruption of the vascular endothelial-cadherin complex at endothelial cell-to-cell junctions. *J. Exp. Med.* **186**, 517-527.

Alon, R., Kassner, P. D., Carr, M. W., Finger, E. B., Hemler, M. E. and Springer, T. A. (1995). The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J. Cell Biol.* **128**, 1243-1253.

Anderson, A. O. and Anderson, N. D. (1976). Lymphocyte emigration from high endothelial venules in rat lymph nodes. *Immunology* **31**, 731-748.

Anderson, J. M., Balda, M. S. and Fanning, A. S. (1993). The structure and regulation of tight junctions. *Curr. Opin. Cell Biol.* **5**, 772-778.

Anderson, N. D., Anderson, A. O. and Wyllie, R. G. (1976). Specialized structure and metabolic activities of high endothelial venules in rat lymphatic tissues. *Immunology* **31**, 455-473.

Ayalon, O., Sabanai, H., Lampugnani, M. G., Dejana, E. and Geiger, B. (1994). Spatial and temporal relationships between cadherins and PECAM-1 in cell-cell junctions of human endothelial cells. *J. Cell Biol.* **126**, 247-258.

Baggiolini, M., Dewald, B. and Moser, B. (1994). Interleukin-8 and related chemotactic cytokines-CXC and CC chemokines. *Advan. Immunol.* **55**, 97-179.

Baggiolini, M. (1998). Chemokines and leukocyte traffic. *Nature* **392**, 565-568.

Bargatze, R. F., Jutila, M. A. and Butcher, E. C. (1995). Distinct roles of L-selectin and integrins alpha 4 beta 7 and LFA-1 in lymphocyte homing to Peyer's patch-HEV in situ: the multistep model confirmed and refined. *Immunity* **3**, 99-108.

Behrens, J., Vakaet, L., Friis, R., Winterhager, E., Van Roy, F., Mareel, M. M. and Birchmeier, W. (1993). Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/beta-catenin complex in cells transformed with a temperature-sensitive v-SRC gene. *J. Cell Biol.* **120**, 757-766.

Ben-Baruch, A., Michiel, D. F. and Oppenheim, J. J. (1995). Signals and receptors involved in recruitment of inflammatory cells. *J. Biol. Chem.* **270**, 11703-11706.

Berg, E. L., McEvoy, L. M., Berlin, C., Bargatze, R. F. and Butcher, E. C. (1993). L-selectin-mediated lymphocyte rolling on MAdCAM-1 [see comments]. *Nature* **366**, 695-698.

Berman, M. E., Xie, Y. and Muller, W. A. (1996). Roles of platelet/endothelial cell adhesion molecule-1 (PECAM-1, CD31) in natural killer cell transendothelial migration and beta 2 integrin activation. *J. Immunol.* **156**, 1515-1524.

Beyer, E. C. (1993). Gap junctions. *Int. Rev. Cytol.* 1-37.

Bianchi, E., Bender, J. R., Blasi, F. and Pardi, R. (1997). Through and beyond the wall: late steps in leukocyte transendothelial migration. *Immunol. Today* **18**, 586-591.

Bjerknes, M., Cheng, H. and Ottaway, C. A. (1986). Dynamics of lymphocyte-endothelial interactions in vivo. *Science* **231**, 402-405.

Bowman, P. D., du Bois, M., Shivers, R. R. and Dorovini-Zis. (1991). Endothelial tight junctions. In *Tight Junctions* (ed. M. Cereijido), pp. 305-320. Florida: CRC Press Inc.

Brown, J. D. and Moon, R. T. (1998). Wnt signaling: why is everything so negative? *Curr. Opin. Cell Biol.* **10**, 182-187.

Buckley, C. D., Doyonnas, R., Newton, J. P., Blystone, S. D., Brown, E. J., Watt, S. M. and Simmons, D. L. (1996). Identification of alpha v beta 3 as a heterotypic ligand for CD31/PECAM-1. *J. Cell Sci.* **109**, 437-445.

Burns, A. R., Walker, D. C., Brown, E. S., Thurmon, L. T., Bowden, R. A., Keese, C. R., Simon, S. I., Entman, M. L. and Smith, C. W. (1997). Neutrophil transendothelial migration is independent of tight junctions and occurs preferentially at tricellular corners. *J. Immunol.* **159**, 2893-2903.

Burns, A. R., Bowden, R. A., MacDonell, S. D., Walker, D. C., Odebunmi, T. O., Donnachie, E. M., Simon, S. I., Entman, M. L. and Smith, C. W. (2000). Analysis of tight junctions during neutrophil transendothelial migration. *J. Cell Sci.* **113**, 45-57.

Butcher, E. C., Williams, M., Youngman, K., Rott, L. and Briskin, M. (1999). Lymphocyte trafficking and regional immunity. *Advan. Immunol.* **72**, 209-253.

Campbell, J. J., Hedrick, J., Zlotnik, A., Siani, M. A., Thompson, D. A. and Butcher, E. C. (1998). Chemokines and the arrest of lymphocytes rolling under flow conditions. *Science* **279**, 381-384.

Carden, D., Xiao, F., Moak, C., Willis, B. H., Robinson-Jackson, S. and Alexander, S. (1998). Neutrophil elastase promotes lung microvascular injury and proteolysis of endothelial cadherins. *Am. J. Physiol.* **275**, H385-392.

Carlos, T. M., Schwartz, B. R., Kovach, N. L., Yee, E., Rosa, M., Osborn, L., Chi-Rosso, G., Newman, B., Lobb, R., Rosso, M. et al. (1990). Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells [published erratum appears in *Blood* **76**, 2420]. *Blood* **76**, 965-970.

Carlos, T. M. and Harlan, J. M. (1994). Leukocyte-endothelial adhesion molecules. *Blood* **84**, 2068-2101.

Carr, M. W., Alon, R. and Springer, T. A. (1996). The C-C chemokine MCP-1 differentially modulates the avidity of β1 and β2 integrins on T lymphocytes. *Immunity* **4**, 179-187.

Cepinskas, G., Sandig, M. and Kvietys, P. R. (1999). PAF-induced elastase-dependent neutrophil transendothelial migration is associated with the mobilization of elastase to the neutrophil surface and localization to the migrating front. *J. Cell Sci.* **112**, 1937-1945.

Chiba, R., Nakagawa, N., Kurasawa, K., Tanaka, Y., Saito, Y. and Iwamoto, I. (1999). Ligation of CD31 (PECAM-1) on endothelial cells increases adhesive function of alphavbeta3 integrin and enhances beta1 integrin-mediated adhesion of eosinophils to endothelial cells. *Blood* **94**, 1319-1329.

Corada, M., Mariotti, M., Thurston, G., Smith, K., Kunkel, R., Brockhaus, M., Lampugnani, M. G., Martin-Padura, I., Stoppacciaro, A., Ruco, L. et al. (1999). Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo. *Proc. Nat. Acad. Sci. USA* **96**, 9815-9820.

Dejana, E., Corada, M. and Lampugnani, M. G. (1995). Endothelial cell-to-cell junctions. *FASEB J.* **9**, 910-918.

Del Maschio, A., Zanetti, A., Corada, M., Rival, Y., Ruco, L., Lampugnani, M. G. and Dejana, E. (1996). Polymorphonuclear leukocyte adhesion triggers the disorganization of endothelial cell-to-cell adherens junctions. *J. Cell Biol.* **135**, 497-510.

DeLisser, H. M., Newman, P. J. and Albelda, S. M. (1994). Molecular and functional aspects of PECAM-1/CD31. *Immunol. Today* **15**, 490-495.

DeLisser, H. M., Christofidou-Solomidou, M., Strieter, R. M., Burdick, M. D., Robinson, C. S., Wexler, R. S., Kerr, J. S., Garlanda, C., Merwin, J. R., Madri, J. A. et al. (1997). Involvement of endothelial PECAM-1/CD31 in angiogenesis. *Am. J. Pathol.* **151**, 671-677.

Doukas, J. and Pober, J. S. (1990). IFN-gamma enhances endothelial activation induced by tumor necrosis factor but not IL-1. *J. Immunol.* **145**, 1727-1733.

Dransfield, I. and Hogg, N. (1989). Regulated expression of Mg^{2+} binding epitope on leukocyte integrin alpha subunits. *EMBO J.* **8**, 3759-3765.

Drayson, M. T. and Ford, W. L. (1984). Afferent lymph and lymph borne cells: their influence on lymph node function. *Immunobiology* **168**, 362-379.

Duncan, G. S., Andrew, D. P., Takimoto, H., Kaufman, S. A., Yoshida, H., Spellberg, J., Luis de la Pompa, J., Elia, A., Wakeham, A., Karan-Tamir, B. et al. (1999). Genetic evidence for functional redundancy of platelet/endothelial cell adhesion molecule-1 (PECAM-1): CD31-deficient

mice reveal PECAM-1-dependent and PECAM-1-independent functions. *J. Immunol.* **162**, 3022-3030.

Dustin, M. L., Rothlein, R., Bhan, A. K., Dinarello, C. A. and Springer, T. A. (1986). Induction by IL 1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J. Immunol.* **137**, 245-254.

Dustin, M. L. and Springer, T. A. (1989). T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* **341**, 619-624.

Esser, S., Lampugnani, M. G., Corada, M., Dejana, E. and Risau, W. (1998). Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. *J. Cell Sci.* **111**, 1853-1865.

Evans, S. S., Collea, R. P., Appenheimer, M. M. and Gollnick, S. O. (1993). Interferon-alpha induces the expression of the L-selectin homing receptor in human B lymphoid cells. *J. Cell Biol.* **123**, 1889-1898.

Feng, D., Nagy, J. A., Pyne, K., Dvorak, H. F. and Dvorak, A. M. (1998). Neutrophils emigrate from venules by a transendothelial cell pathway in response to FMLP. *J. Exp. Med.* **187**, 903-915.

Furuse, M., Hirase, T., Itoh, M., Nagafuchi, A., Yonemura, S. and Tsukita, S. (1993). Occludin: a novel integral membrane protein localizing at tight junctions [see comments]. *J. Cell Biol.* **123**, 1777-1788.

Furuse, M., Fujita, K., Hiiragi, T., Fujimoto, K. and Tsukita, S. (1998). Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J. Cell Biol.* **141**, 1539-1550.

Geiger, B. and Ayalon, O. (1992). Cadherins. *Annu. Rev. Cell Biol.* **8**, 307-332.

Ginsberg, M. H., Du, X. and Plow, E. F. (1992). Inside-out integrin signalling. *Curr. Opin. Cell Biol.* **4**, 766-771.

Girard, J. P. and Springer, T. A. (1995). High endothelial venules (HEV): specialized endothelium for lymphocyte migration. *Immunol. Today* **16**, 449-457.

Goeckeler, Z. M. and Wysolmerski, R. B. (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.

Gowans, J. L. and Knight, E. J. (1964). The route of recirculation of lymphocytes. *Proc. R. Soc. Lon. Ser. B* **159**, 257.

Grant, L. (1973). The sticking and emigration of white blood cells in inflammation. In *The Inflammatory Process*, vol. 11 (ed. B. Zweifach, L. Grant and R. McCluskey), pp. 205-249. New York: Academic Press.

Griffin, J. D., Spertini, O., Ernst, T. J., Belvin, M. P., Levine, H. B., Kanakura, Y. and Tedder, T. F. (1990). Granulocyte-macrophage colony-stimulating factor and other cytokines regulate surface expression of the leukocyte adhesion molecule-1 on human neutrophils, monocytes, and their precursors. *J. Immunol.* **145**, 576-584.

Gumbiner, B. M. (1993). Breaking through the tight junction barrier [comment]. *J. Cell Biol.* **123**, 1631-1633.

Gumbiner, B. M. (1996). Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell* **84**, 345-357.

Hall, J. G. and Morris, B. (1965). The origin of cells in the efferent lymph from a single lymph node. *J. Exp. Med.* **121**, 901-910.

Hattori, R., Hamilton, K. K., Fugate, R. D., McEver, R. P. and Sims, P. J. (1989). Stimulated secretion of endothelial von Willebrand factor is accompanied by rapid redistribution to the cell surface of the intracellular granule membrane protein GMP-140. *J. Biol. Chem* **264**, 7768-7771.

Hedrick, J. A. and Zlotnik, A. (1996). Chemokines and lymphocyte biology. *Curr. Opin. Immunol.* **8**, 343-347.

Hendriks, H. R. and Eestermans, I. L. (1983). Disappearance and reappearance of high endothelial venules and immigrating lymphocytes in lymph nodes deprived of afferent lymphatic vessels: a possible regulatory role of macrophages in lymphocyte migration. *Eur J. Immunol.* **13**, 663-669.

Hixenbaugh, E. A., Goeckeler, Z. M., Papaiya, N. N., Wysolmerski, R. B., Silverstein, S. C. and Huang, A. J. (1997). Stimulated neutrophils induce myosin light chain phosphorylation and isometric tension in endothelial cells. *Am. J. Physiol.* **273**, H981-988.

Hordijk, P. L., Anthony, E., Mul, F. P., Rientsma, R., Oomen, L. C. and Roos, D. (1999). Vascular-endothelial-cadherin modulates endothelial monolayer permeability. *J. Cell Sci.* **112**, 1915-1923.

Hu, M. C., Holzmann, B., Crowe, D. T., Neuhaus, H. and Weissman, I. L. (1993). The Peyer's patch homing receptor. *Curr. Top. Microbiol. Immunol.* **184**, 125-138.

Huang, A. J., Furie, M. B., Nicholson, S. C., Fischbarg, J., Liebovitch, L. S. and Silverstein, S. C. (1988). Effects of human neutrophil chemotaxis across human endothelial cell monolayers on the permeability of these monolayers to ions and macromolecules. *J. Cell Physiol.* **135**, 355-366.

Huang, A. J., Manning, J. E., Bandak, T. M., Ratau, M. C., Hanser, K. R. and Silverstein, S. C. (1993). Endothelial cell cytosolic free calcium regulates neutrophil migration across monolayers of endothelial cells. *J. Cell Biol.* **120**, 1371-1380.

Hurley, J. (1983). *Acute Inflammation*. Edinburgh: Churchill Livingstone.

Hwang, S. T., Singer, M. S., Giblin, P. A., Yednock, T. A., Bacon, K. B., Simon, S. I. and Rosen, S. D. (1996). GlyCAM-1, a physiologic ligand for L-selectin, activates beta 2 integrins on naive peripheral lymphocytes. *J. Exp. Med.* **184**, 1343-1348.

Hynes, R. O. (1992). Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11-25.

Ikeda, S., Kishida, S., Yamamoto, H., Murai, H., Koyama, S. and Kikuchi, A. (1998). Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. *EMBO J.* **17**, 1371-1384.

Ilan, N., Mahooti, S., Rimm, D. L. and Madri, J. A. (1999). PECAM-1 (CD31) functions as a reservoir for and a modulator of tyrosine-phosphorylated β-catenin. *J. Cell Sci.* **112**, 3005-3014.

Imhof, B. A. and Dunon, D. (1995). Leukocyte migration and adhesion. *Advan. Immunol.* **58**, 345-416.

Kemler, R. (1993). From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet.* **9**, 317-321.

Kraal, G. and Mebius, R. E. (1997). High endothelial venules: lymphocyte traffic control and controlled traffic. *Advan. Immunol.* **65**, 347-395.

Kucik, D. F., Dustin, M. L., Miller, J. M. and Brown, E. J. (1996). Adhesion-activating phorbol ester increases the mobility of leukocyte integrin LFA-1 in cultured lymphocytes. *J. Clin. Invest.* **97**, 2139-2144.

Kuijpers, T. W., Hakkert, B. C., Hoogerwerf, M., Leeuwenberg, J. F. and Roos, D. (1991). Role of endothelial leukocyte adhesion molecule-1 and platelet-activating factor in neutrophil adherence to IL-1-prestimulated endothelial cells. Endothelial leukocyte adhesion molecule-1-mediated CD18 activation. *J. Immunol.* **147**, 1369-1376.

Lampugnani, M. G., Resnati, M., Raiteri, M., Pigott, R., Pisacane, A., Houen, G., Ruco, L. P. and Dejana, E. (1992). A novel endothelial-specific membrane protein is a marker of cell-cell contacts. *J. Cell Biol.* **118**, 1511-1522.

Lampugnani, M. G., Corada, M., Caveda, L., Breviario, F., Ayalon, O., Geiger, B. and Dejana, E. (1995). The molecular organization of endothelial cell to cell junctions: differential association of plakoglobin, beta-catenin, and alpha-catenin with vascular endothelial cadherin (VE-cadherin). *J. Cell Biol.* **129**, 203-217.

Larsen, G. L., McCarthy, K., Webster, R. O., Henson, J. and Henson, P. M. (1980). A differential effect of C5a and C5a des Arg in the induction of pulmonary inflammation. *Am. J. Pathol.* **100**, 179-192.

Larson, R. S. and Springer, T. A. (1990). Structure and function of leukocyte integrins. *Immunol. Rev.* **114**, 181-217.

Lasky, L. A. (1992). Selectins: Interpreters of cell-specific carbohydrate information during inflammation. *Science* **258**, 964-969.

Lasky, L. A. (1995). Selectin-carbohydrate interactions and the initiation of the inflammatory response. *Annu. Rev. Biochem.* **64**, 113-139.

Leavesley, D. I., Oliver, J. M., Swart, B. W., Berndt, M. C., Haylock, D. N., Simmons, P. J. (1994). Signals from platelet/endothelial cell adhesion moelcule enhance the adhesive activity of the very late antigen-4 integrin of human CD34+ hemopoietic progenitor cells. *J. Immunol.* **153**, 4673.

Leeuwenberg, J. F., von Asmuth, E. J., Jeunhomme, T. M. and Buurman, W. A. (1990). IFN-gamma regulates the expression of the adhesion molecule ELAM-1 and IL-6 production by human endothelial cells in vitro. *J. Immunol.* **145**, 2110-2114.

Ley, K. and Tedder, T. F. (1995). Leukocyte interactions with vascular endothelium. New insights into selectin-mediated attachment and rolling. *J. Immunol.* **155**, 525-528.

Lloyd, A. R., Oppenheim, J. J., Kelvin, D. J. and Taub, D. D. (1996). Chemokines regulate T cell adherence to recombinant adhesion molecules and extracellular matrix proteins. *J. Immunol.* **156**, 932-938.

Lo, S. K., Lee, S., Ramos, R. A., Lobb, R., Rosa, M., Chi-Rosso, G. and Wright, S. D. (1991). Endothelial-leukocyte adhesion molecule 1 stimulates the adhesive activity of leukocyte integrin CR3 (CD11b/CD18, Mac-1, alpha m beta 2) on human neutrophils. *J. Exp. Med.* **173**, 1493-1500.

Lobb, R. R. and Hemler, M. E. (1994). The pathophysiologic role of alpha 4 integrins in vivo. *J. Clin. Invest.* **94**, 1722-1728.

Mackay, C. R., Marston, W. L. and Dudler, L. (1990). Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J. Exp. Med.* **171**, 801-817.

Malergue, F., Galland, F., Martin, F., Mansuelle, P., Aurrand-Lions, M.

and Naquet, P. (1998). A novel immunoglobulin superfamily junctional molecule expressed by antigen presenting cells, endothelial cells and platelets. *Mol. Immunol.* **35**, 1111-1119.

Mantovani, A., Bussolino, F. and Introna, M. (1997). Cytokine regulation of endothelial cell function: from molecular level to the bedside. *Immunol. Today* **18**, 231-240.

Mantovani, A., Allavena, P., Vecchi, A. and Sozzani, S. (1998). Chemokines and chemokine receptors during activation and deactivation of monocytes and dendritic cells and in amplification of Th1 versus Th2 responses. *Int. J. Clin. Lab. Res.* **28**, 77-82.

Mantovani, A. (1999). The chemokine system: redundancy for robust outputs. *Immunol. Today* **20**, 254-257.

Marchesi, V. T. and Gowans, J. L. (1964). *Proc. R. Soc. Lon. Ser. B* **159**, 283-290.

Martin-Padura, I., Lostaglio, S., Schneemann, M., Williams, L., Romano, M., Fruscella, P., Panzeri, C., Stoppacciaro, A., Ruco, L., Villa, A. et al. (1998). Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J. Cell Biol.* **142**, 117-127.

Masinovsky, B., Urdal, D. and Gallatin, W. M. (1990). IL-4 acts synergistically with IL-1 beta to promote lymphocyte adhesion to microvascular endothelium by induction of vascular cell adhesion molecule-1. *J. Immunol.* **145**, 2886-2895.

Matsumura, T., Wolff, K. and Petzelbauer, P. (1997). Endothelial cell tube formation depends on cadherin 5 and CD31 interactions with filamentous actin. *J. Immunol.* **158**, 3408-3416.

Matsuyoshi, N., Hamaguchi, M., Taniguchi, S., Nagafuchi, A., Tsukita, S. and Takeichi, M. (1992). Cadherin-mediated cell-cell adhesion is perturbed by v-src tyrosine phosphorylation in metastatic fibroblasts. *J. Cell Biol.* **118**, 703-714.

McEver, R. P., Beckstead, J. H., Moore, K. L., Marshall-Carlson, L. and Bainton, D. F. (1989). GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. *J. Clin. Invest.* **84**, 92-99.

Moll, T., Dejana, E. and Vestweber, D. (1998). In vitro degradation of endothelial catenins by a neutrophil protease. *J. Cell Biol.* **140**, 403-407.

Moore, K. L., Patel, K. D., Bruehl, R. E., Li, F., Johnson, D. A., Lichenstein, H. S., Cummings, R. D., Bainton, D. F. and McEver, R. P. (1995). P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. *J. Cell Biol.* **128**, 661-671.

Moy, A. B., Shasby, S. S., Scott, B. D. and Shasby, D. M. (1993). The effect of histamine and cyclic adenosine monophosphate on myosin light chain phosphorylation in human umbilical vein endothelial cells. *J. Clin. Invest.* **92**, 1198-1206.

Moy, A. B., Van Engelenhoven, J., Bodmer, J., Kamath, J., Keese, C., Giaever, I., Shasby, S. and Shasby, D. M. (1996). Histamine and thrombin modulate endothelial focal adhesion through centripetal and centrifugal forces. *J. Clin. Invest.* **97**, 1020-1027.

Muller, W. A., Berman, M. E., Newman, P. J., DeLisser, H. M. and Albelda, S. M. (1992). A heterophilic adhesion mechanism for platelet/endothelial cell adhesion molecule 1 (CD31). *J. Exp. Med.* **175**, 1401-1404.

Muller, W. A., Weigl, S. A., Deng, X. and Phillips, D. M. (1993). PECAM-1 is required for transendothelial migration of leukocytes. *J. Exp. Med.* **178**, 449-460.

Nagel, T., Resnick, N., Atkinson, W. J., Dewey, C. F. Jr and Gimbrone, M. A. Jr (1994). Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. *J. Clin. Invest.* **94**, 885-891.

Nakache, M., Berg, E. L., Streeter, P. R. and Butcher, E. C. (1989). The mucosal vascular addressin is a tissue-specific endothelial cell adhesion molecule for circulating lymphocytes. *Nature* **337**, 179-181.

Newton, J. P., Buckley, C. D., Jones, E. Y. and Simmons, D. L. (1997). Residues on both faces of the first immunoglobulin fold contribute to homophilic binding sites of PECAM-1/CD31. *J. Biol. Chem.* **272**, 20555-20563.

Ozaki, H., Ishii, K., Horiuchi, H., Arai, H., Kawamoto, T., Okawa, K., Iwamatsu, A. and Kita, T. (1999). Cutting edge: combined treatment of TNF-alpha and IFN-gamma causes redistribution of junctional adhesion molecule in human endothelial cells. *J. Immunol.* **163**, 553-557.

Pabst, R. and Binns, R. M. (1989). Heterogeneity of lymphocyte homing physiology: several mechanisms operate in the control of migration to lymphoid and non-lymphoid organs in vivo. *Immunol. Rev.* **108**, 83-109.

Pfau, S., Leitenberg, D., Rinder, H., Smith, B. R., Pardi, R. and Bender, J. R. (1995). Lymphocyte adhesion-dependent calcium signaling in human endothelial cells. *J. Cell Biol.* **128**, 969-978.

Piali, L., Albelda, S. M., Baldwin, H. S., Hammel, P., Gisler, R. H. and Imhof, B. A. (1993). Murine platelet endothelial cell adhesion molecule (PECAM-1)/CD31 modulates beta 2 integrins on lymphokine-activated killer cells. *Eur. J. Immunol.* **23**, 2464-2471.

Piali, L., Hammel, P., Uherek, C., Bachmann, F., Gisler, R. H., Dunon, D. and Imhof, B. A. (1995). CD31/PECAM-1 is a ligand for alpha v beta 3 integrin involved in adhesion of leukocytes to endothelium. *J. Cell Biol.* **130**, 451-460.

Picker, L. J., Warnock, R. A., Burns, A. R., Doerschuk, C. M., Berg, E. L. and Butcher, E. C. (1991). The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140. *Cell* **66**, 921-933.

Picker, L. J., Treer, J. R., Ferguson-Darnell, B., Collins, P. A., Bergstresser, P. R. and Terstappen, L. W. M. M. (1993). Control of lymphocyte recirculation in man: II. Differential regulation of the cutaneous lymphocyte-associated antigen (CLA), a tissue-selective homing receptor for skin-homing T cells. *J. Immunol.* (in press).

Pober, J. S. and Cotran, R. S. (1990). The role of endothelial cells in inflammation. *Transplantation* **50**, 537-544.

Prager, E., Sunder Plassmann, R., Hansmann, C., Koch, C., Holter, W., Knapp, W. and Stockinger, H. (1996). Interaction of CD31 with a heterophilic counterreceptor involved in downregulation of human T cell responses. *J. Exp. Med.* **184**, 41-50.

Rabiet, M. J., Plantier, J. L. and Dejana, E. (1994). Thrombin-induced endothelial cell dysfunction. *Br. Med. Bull.* **50**, 936-945.

Rabiet, M. J., Plantier, J. L., Rival, Y., Genoux, Y., Lampugnani, M. G. and Dejana, E. (1996). Thrombin-induced increase in endothelial permeability is associated with changes in cell-to-cell junction organization. *Arterioscler. Thromb. Vasc. Biol.* **16**, 488-496.

Rival, Y., Del Maschio, A., Rabiet, M. J., Dejana, E. and Duperray, A. (1996). Inhibition of platelet endothelial cell adhesion molecule-1 synthesis and leukocyte transmigration in endothelial cells by the combined action of TNF-alpha and IFN-gamma. *J. Immunol.* **157**, 1233-1241.

Rosen, S. D. (1999). Selectins. In *Guide to the Extracellular Matrix and Adhesion Proteins* (ed. R. Vale and T. Kreis), pp. 290-297. Oxford: Oxford University Press.

Rosen, S. D. and Bertozzi, C. R. (1994). The selectins and their ligands. *Curr. Opin. Cell Biol.* **6**, 663-673.

Rubin, L. L. (1992). Endothelial cells: adhesion and tight junctions. *Curr. Opin. Cell Biol.* **4**, 830-833.

Saito, H., Minamiya, Y., Kitamura, M., Saito, S., Enomoto, K., Terada, K. and Ogawa, J. (1998). Endothelial myosin light chain kinase regulates neutrophil migration across human umbilical vein endothelial cell monolayer. *J. Immunol.* **161**, 1533-1540.

Sakanaka, C., Weiss, J. B. and Williams, L. T. (1998). Bridging of beta-catenin and glycogen synthase kinase-3beta by axin and inhibition of beta-catenin-mediated transcription. *Proc. Nat. Acad. Sci. USA* **95**, 3020-3023.

Salmi, M. and Jalkanen, S. (1997). How do lymphocytes know where to go: current concepts and enigmas of lymphocyte homing. *Advan. Immunol.* **64**, 139-218.

Schmelz, M. and Franke, W. W. (1993). Complexus adhaerentes, a new group of desmoplakin-containing junctions in endothelial cells: the syndesmos connecting retothelial cells of lymph nodes. *Eur. J. Cell Biol.* **61**, 274-289.

Sheldon, R., Moy, A., Lindsley, K., Shasby, S. and Shasby, D. M. (1993). Role of myosin light-chain phosphorylation in endothelial cell retraction. *Am. J. Physiol.* **265**, L606-612.

Shimizu, Y., Rose, D. M. and Ginsberg, M. H. (1999). Integrins in the immune system. *Advan. Immunol.* **72**, 325-380.

Shivers, R. R., Arthur, F. E. and Bowman, P. D. (1988). Induction of gap junctions and brain endothelium-like tight junctions in cultured bovine endothelial cells: local control of cell specialization. *J. Submicrosc. Cytol. Pathol.* **20**, 1-14.

Simionescu, M. and Simionescu, N. (1991). Endothelial transport of macromolecules: transcytosis and endocytosis. A look from cell biology. *Cell Biol. Rev.* **25**, 5-78.

Simionescu, M., Simionescu, N. and Palade, G. E. (1976). Segmental differentiations of cell junctions in the vascular endothelium. Arteries and veins. *J. Cell Biol.* **68**, 705-723.

Simon, S. I., Burns, A. R., Taylor, A. D., Gopalan, P. K., Lynam, E. B., Sklar, L. A. and Smith, C. W. (1995). L-selectin (CD62L) cross-linking

signals neutrophil adhesive functions via the Mac-1 (CD11b/CD18) β2-integrin. *J. Immunol.* **155**, 1502-1514.

Sironi, M., Sciacca, F. L., Matteucci, C., Conni, M., Vecchi, A., Bernasconi, S., Minty, A., Caput, D., Ferrara, P., Colotta, F. et al. (1994). Regulation of endothelial and mesothelial cell function by interleukin-13: selective induction of vascular cell adhesion molecule-1 and amplification of interleukin-6 production. *Blood* **84**, 1913-1921.

Springer, T. A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* **76**, 301-314.

Stewart, M., Thiel, M. and Hogg, N. (1995). Leukocyte integrins. *Curr. Opin. Cell Biol.* **7**, 690-696.

Stoolman, L. M. (1989). Adhesion molecules controlling lymphocyte migration. *Cell* **56**, 907-910.

Streeter, P. R., Berg, E. L., Rouse, B. T., Bargatze, R. F. and Butcher, E. C. (1988). A tissue-specific endothelial cell molecule involved in lymphocyte homing. *Nature* **331**, 41-46.

Sun, Q. H., DeLisser, H. M., Zukowski, M. M., Paddock, C., Albelda, S. M. and Newman, P. J. (1996). Individually distinct Ig homology domains in PECAM-1 regulate homophilic binding and modulate receptor affinity. *J. Biol. Chem.* **271**, 11090-11098.

Takeichi, M. (1991). Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* **251**, 1451-1455.

Tanaka, Y., Albelda, S. M., Horgan, K. J., van Seventer, G. A., Shimizu, Y., Newman, W., Hallam, J., Newman, P. J., Buck, C. A. and Shaw, S. (1992). CD31 expressed on distinctive T cell subsets is a preferential amplifier of beta 1 integrin-mediated adhesion. *J. Exp. Med.* **176**, 245-253.

Tanaka, Y., Adams, D. H., Hubscher, S., Hirano, H., Siebenlist, U. and Shaw, S. (1993). T-cell adhesion induced by proteoglycan-immobilized cytokine MIP-1 beta. *Nature* **361**, 79-82.

Taub, D. D., Conlon, K., Lloyd, A. R., Oppenheim, J. J. and Kelvin, D. J. (1993a). Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1α and MIP-1β. *Science* **260**, 355-358.

Taub, D. D., Lloyd, A. R., Conlon, K., Wang, J. M., Ortaldo, J. R., Harada, A., Matsushima, K., Kelvin, D. J. and Oppenheim, J. J. (1993b). Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. *J. Exp. Med.* **177**, 1809-1814.

Thornhill, M. H. and Haskard, D. O. (1990). IL-4 regulates endothelial cell activation by IL-1, tumor necrosis factor, or IFN-gamma. *J. Immunol.* **145**, 865-872.

Tinsley, J. H., Wu, M. H., Ma, W., Taulman, A. C. and Yuan, S. Y. (1999). Activated neutrophils induce hyperpermeability and phosphorylation of adherens junction proteins in coronary venular endothelial cells. *J. Biol. Chem.* **274**, 24930-24934.

Tio, S., Deenen, M. and Marani, E. (1990). Astrocyte-mediated induction of alkaline phosphatase activity in human umbilical cord vein endothelium: an in vitro model. *Eur. J. Morphol.* **28**, 289-300.

Tsukita, S., Nagafuchi, A. and Yonemura, S. (1992). Molecular linkage between cadherins and actin filaments in cell-cell adherens junctions. *Curr. Opin. Cell Biol.* **4**, 834-839.

Tu, L., Delahunty, M. D., Ding, H., Luscinskas, F. W. and Tedder, T. F. (1999). The cutaneous lymphocyte antigen is an essential component of the L-selectin ligand induced on human vascular endothelial cells. *J. Exp. Med.* **189**, 241-252.

van Furth, R. (1986). Current view on the mononuclear phagocyte system. *Immunobiology* **161**, 178.

van Kooyk, Y., van de Wiel-van Kemade, P., Weder, P., Kuijpers, T. W. and Figdor, C. G. (1989). Enhancement of LFA-1-mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. *Nature* **342**, 811-813.

van Kooyk, Y., Weder, P., Heije, K. and Figdor, C. G. (1994). Extracellular Ca2+ modulates leukocyte function-associated antigen-1 cell surface distribution on T lymphocytes and consequently affects cell adhesion. *J. Cell Biol.* **124**, 1061-1070.

Varon, D., Jackson, D. E., Shenkman, B., Dardik, R., Tamarin, I., Savion, N. and Newman, P. J. (1998). Platelet/endothelial cell adhesion molecule-1 serves as a costimulatory agonist receptor that modulates integrin-dependent adhesion and aggregation of human platelets. *Blood* **91**, 500-507.

Vestweber, D. (1993). The selectins and their ligands. *Curr. Top. Microbiol. Immunol.* **184**, 65-75.

Walker, D. C., MacKenzie, A. and Hosford, S. (1994). The structure of the tricellular region of endothelial tight junctions of pulmonary capillaries analyzed by freeze-fracture. *Microvasc. Res.* **48**, 259-281.

Watt, S. M., Gschmeissner, S. E. and Bates, P. A. (1995). PECAM-1: its expression and function as a cell adhesion molecule on hemopoietic and endothelial cells. *Leuk Lymphoma* **17**, 229-244.

Wellicome, S. M., Thornhill, M. H., Pitzalis, C., Thomas, D. S., Lanchbury, J. S., Panayi, G. S. and Haskard, D. O. (1990). A monoclonal antibody that detects a novel antigen on endothelial cells that is induced by tumor necrosis factor, IL-1, or lipopolysaccharide. *J. Immunol.* **144**, 2558-2565.

Westermann, J. and Pabst, R. (1990). Lymphocyte subsets in the blood: a diagnostic window on the lymphoid system? [see comments]. *Immunol. Today* **11**, 406-410.

Westermann, J. and Pabst, R. (1996). How organ-specific is the migration of 'naive' and 'memory' T cells? [see comments]. *Immunol. Today* **17**, 278-282.

Wolburg, H., Neuhaus, J., Kniesel, U., Krauss, B., Schmid, E. M., Ocalan, M., Farrell, C. and Risau, W. (1994). Modulation of tight junction structure in blood-brain barrier endothelial cells. Effects of tissue culture, second messengers and cocultured astrocytes. *J. Cell Sci.* **107**, 1347-1357.

Yee, A. G. and Revel, J. P. (1975). Endothelial cell junctions. *J. Cell Biol.* **66**, 200-204.

Yuan, Y., Meng, F. Y., Huang, Q., Hawker, J. and Wu, H. M. (1998). Tyrosine phosphorylation of paxillin/pp125FAK and microvascular endothelial barrier function. *Am. J. Physiol.* **275**, H84-93.