Migration of human hematopoietic progenitor cells

Voermans, C.

Citation for published version (APA):
Chapter 6

Migration of human hematopoietic progenitor cells across bone-marrow endothelium is regulated by vascular-endothelial cadherin.

Submitted for publication
Migration of human hematopoietic progenitor cells across bone-marrow endothelium is regulated by vascular-endothelial cadherin.

Jaap D. van Buul¹, Carlijn Voermans¹,², Veronique van den Berg¹, Eloise C. Anthony¹, C. Ellen van der Schoot¹,³, and Peter L. Hordijk¹

¹Department of Experimental Immunohematology, CLB and Laboratory for Experimental and Clinical Immunology, Academic Medical Center, University of Amsterdam, The Netherlands
²Division of Medical Oncology, Netherlands Cancer Institute, Amsterdam. The Netherlands
³Department of Hematology, Academic Medical Center, Amsterdam, The Netherlands

ABSTRACT

The success of stem cell transplantation depends on the ability of intravenously infused stem cells to engraft the bone marrow, a process referred to as homing. Efficient homing requires migration of CD34⁺ cells across the intercellular junctions of the bone-marrow endothelium. The adhesion between endothelial cells is controlled by VE-cadherin. Here we show that loss of VE-cadherin function increases the permeability of monolayers of human bone marrow endothelial cells (HBMEC) and stimulates the transendothelial migration of CD34⁺ cells in response to SDF-1α. The SDF-1α-induced migration was dependent on ICAM-1 and VCAM-1, even in the absence of VE-cadherin function. Crosslinking of ICAM-1, to mimic the leukocyte-endothelium interaction, induced actin stress fiber formation and reduced the HBMEC permeability, whereas crosslinking of VCAM-1 increased the HBMEC permeability and induced discernible gaps in the monolayer. These data suggest that modulation of VE-cadherin function directly affects the efficiency of transendothelial migration of CD34⁺ cells. The endothelial adhesion molecules ICAM-1 and in particular VCAM-1 play an important role in this process by activating intracellular signaling that regulates the integrity of the bone-marrow endothelium.
INTRODUCTION

Hematopoietic stem cell transplantation is applied to restore hematopoiesis in cancer patients after myelo-ablative chemotherapy and/or after irradiation. The success of the transplantation depends on the ability of the hematopoietic stem cells to engraft the bone marrow, a process referred to as homing [1]. An important step in homing is the actual transmigration of reinfused stem cells across the bone-marrow endothelium to the bone-marrow stroma. Whereas much is known about the migration of granulocytes and T-cells, the factors that control the transendothelial migration of hematopoietic stem cells are still poorly understood.

Recently, the first powerful chemotactant for hematopoietic stem cells (CD34+ cells) has been described, i.e. stromal cell-derived factor-1α (SDF-1α), produced by several types of stromal cell, including those of the bone marrow [2-5]. SDF-1α signals through a G-protein-coupled receptor, called Fusin, LESTR or CXCR-4 [6-9]. SDF-1α-driven homing of CD34+ cells has been suggested to be a multi-step process similar to the extravasation process of leukocytes at inflammatory sites [10] and is mediated by adhesion molecules both on CD34+ cells [11] and on bone-marrow endothelial cells. In the final stage of homing, CD34+ cells migrate across the bone-marrow endothelium, presumably via the intercellular junctions. Therefore, endothelial cell-cell adhesion is most likely an important regulatory factor in the homing of CD34+ cells.

Endothelial cell-cell adhesion is largely dependent on the homotypic cell-cell adhesion molecule vascular-endothelial-cadherin (VE-cadherin, cadherin-5, CD144). VE-cadherin is a transmembrane protein that, like other members of the cadherin-family [12], associates via its cytoplasmic tail with various cytosolic proteins, including α-, β-, and γ-catenin (plakoglobin), and p120/p100. These proteins link VE-cadherin to the cortical actin cytoskeleton [13-15]. The role of VE-cadherin in leukocyte transendothelial migration was first described by Gotsch and colleagues, who showed an accelerated extravasation of neutrophils in a mouse peritonitis model in vivo upon intravenous injection of a monoclonal antibody against mouse VE-cadherin [16]. Transfection experiments and gene-inactivation studies have shown that VE-cadherin expression reduces monolayer permeability, promotes cell aggregation, motility and growth, and that VE-cadherin is required for the organization of vascular-like structures in embryoid bodies [17-19]. Moreover, VE-cadherin, together with β-catenin, seems to be involved in cell survival [20]. Regulation of VE-cadherin, and thereby of endothelial cell-cell adhesion, may occur through tyrosine or serine phosphorylation [21-24].
VE-cadherin modulates migration of HPC

association with regulatory proteins [20], and modulation of the endothelial actin cytoskeleton [17,25]. In addition, several studies have proposed a role for leukocyte-adhesion-induced signaling, e.g. through activation of myosin light chain kinase in endothelial cells, which may indirectly regulate VE-cadherin function in the process of transendothelial migration [26-29].

A role for specific adhesion molecules in endothelial-cell signaling has been suggested by studies on VCAM-1 (CD106) and ICAM-1 (CD54). Lorenzon and colleagues concluded that VCAM-1 and endothelial selectins, in addition to their role as adhesion receptors, mediate endothelial stimulation by adherent leukocytes [30]. Moreover, activation of ICAM-1 on endothelial cells following binding of T-cells has been reported to induce tyrosine phosphorylation of the actin-binding protein cortactin [31]. In line with this, it was suggested that ICAM-1 mediates cell shape changes through coupling to the p21Rho GTPase and by inducing phosphorylation of cytoskeletal proteins and transcription factors [32].

In the present study, the role of VE-cadherin in the control of permeability of bone-marrow endothelium and of the transmigration of CD34+ cells was investigated. For this purpose, we used immortalized human bone-marrow endothelial cells (HBMEC) [33], and CD34+ cells isolated from cord blood or from peripheral blood of healthy volunteers. Using transmigration and permeability assays in combination with analysis of the actin cytoskeleton and cell-cell adhesion, we here show that adhesion of CD34+ cells to HBMEC through VCAM-1 and PECAM-1/CD31 induces reduced VE-cadherin-mediated cell-cell adhesion, which facilitates SDF-1α-driven transendothelial migration.

METHODS

Reagents and Abs.
mAbs to VE-cadherin were from Transduction Laboratories (Becton Dickinson Company, the Netherlands (cl75)) or Pharmingen (San Diego, USA (55-7H1)). Polyclonal Ab to VE-cadherin was from Sanvertech (Heerhugowaard, the Netherlands (C-19)). mAbs to PECAM-1/CD31 were generated in our own institute (CLB, Amsterdam, the Netherlands) and have been described [34]. Recombinant human Interleukin-1β (IL-1β) was from PreproTech (Rocky Hill, N.J., USA); Calcein-acetoxyethyl (-AM), Texas-Red Phalloidin, FITC-Dextran 3000 and FITC-dextran 40000, ALEXA 488-labeled goat-anti-mouse-Ig (GαM-Ig) and ALEXA 488-labeled goat-anti-rabbit-Ig (GαR-Ig) secondary Abs were from
Molecular Probes (Leiden, the Netherlands). PE-labeled secondary Abs and C3 transferase were from DAKO (Glostrup, Denmark). HSA, fibronectin (FN) and control Abs IgG1 and IgG2a were obtained from the CLB. FCS was from Gibco BRL (Life Technologies, Paisley, Scotland, UK). bFGF was from Boehringer Mannheim (Mannheim, Germany). CXCR-4 expression was quantitated with PE-labeled anti-human Fusi (12G5, Pharmingen). mAbs to ICAM-1 (84H10) and VCAM-1 (1G11) were purchased from Immunotech SA (Marseille, France). Crosslinking studies were performed with F(ab)_2 fragments of goat-anti-mouse IgG from Jackson Immunoresearch (Baltimore, MD, USA).

**Isolation of CD34+ hematopoietic progenitor cells.**

Cord blood (CB) was collected after delivery, according to the guidelines of Eurocord, and peripheral blood from healthy volunteers (HV) was obtained from the local bloodbank. Mononuclear leukocytes from whole blood of HV (500 ml) and CB were enriched by density gradient centrifugation over Ficoll-paque (1.077 g/ml) (Pharmacia Biotech, Uppsala, Sweden). Then, the HV mononuclear fraction was purified from thrombocytes by elutriation and further processed, similar to CB CD34+ cell isolation, with the VarioMacs system (Miltenyi Biotec GmbH, Gladbach, Germany) as described [2]. At least 95% of the cells from CB, and >90% of the cells from HV expressed CD34 as determined by FACS with CD34 Ab (no. 581, Immunotech).

**Cell cultures.**

The human bone-marrow endothelial cell line has been described previously [33]. The cells were cultured in FN-coated culture flasks (NUNC, Life Technologies) in Medium 199 (Gibco BRL), supplemented with 10% (v/v) pooled, heat-inactivated human serum (CLB), 10% (v/v) heat-inactivated fetal calf serum (FCS) (Gibco BRL), 1 ng/ml bFGF (Boehringer Mannheim), 5 U/ml heparin (Leo Pharmaceutical Products, Weesp, the Netherlands), 300 µg/ml glutamine (Sigma Chemical Co., St. Louis, USA), 100 U/ml penicillin and 100 µg/ml streptomycin. After reaching confluency, the endothelial cells were passaged by treatment with trypsin/EDTA solution (Gibco BRL). HL-60 and KG-1a cell lines were obtained from ATCC (Rockville, MO, USA) and were maintained in Iscove’s Modified Dulbecco’s Medium (IMDM, Biowhittaker, Brussels, Belgium) containing L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% FCS. All cell
lines were cultured at 37°C at 5% CO₂. In all experiments, HBMEC monolayers were pretreated with IL-1β for 4 hours.

**Permeability assays.**
Permeability of HBMEC monolayers, cultured on 5 μm pore Transwell filters (Costar, Cambridge, MA) of 6.5 mm, was assayed with FITC-labeled 3000 dextran as described [17]. In some experiments, monolayers were pretreated with Abs (10 μg/ml, 1 hour). All reagents were present during the permeability assays. The C3 transferase was added to the HBMEC monolayers 6 hours prior to the assays. After the assays, the filters were fixed and stained with Texas-Red phalloidin to inspect the HBMEC monolayer by confocal laser scanning microscopy (CLSM).

**Transendothelial Migration Assay.**
Transmigratory assays were performed in Transwell plates of 6.5 mm diameter, with 5 μm pore filters. HBMEC were plated at 50,000 cells/Transwell on FN-coated filters. Non-adherent cells were removed after 18 hours. Freshly isolated CD34⁺ cells (20,000-100,000) were added to the upper compartment and the assay was performed as described previously [2]. In blocking experiments, HBMEC were preincubated for 30 minutes at 37°C with mAbs (10 μg/ml), followed by washing. As controls, IgGl and IgG2a isotypes were used. No mAbs were present during the assays. After the assays, the filters were fixed and stained with Texas-Red phalloidin to analyse HBMEC monolayer by CLSM.

**Immunocytochemistry.**
HBMEC were cultured on FN-coated glass coverslips and were fixed and immunostained as described [17] with mAb to VE-cadherin (7H1, 25 μg/ml) followed by staining with fluorescently labeled secondary Abs (20 μg/ml). F-actin was visualised by Texas-Red Phalloidin (1 U/ml). In some experiments, cells were pretreated with mAb to VE-cadherin (cl75, 1 hour, 10 μg/ml). In the analysis following the permeability experiments, a goat polyclonal Ab to VE-cadherin was used for immunostaining. Images were recorded with a ZEISS LSM510 confocal microscope with appropriate filter settings. Crosstalk between the green and red channel was avoided by use of sequential scanning.

**Flow Cytometry.**
The expression of surface antigens on the HBMEC was measured by flow cytometry. Following preincubations, the HBMEC were detached with 5 mM
EDTA in calcium-free HEPES medium for 15 minutes at 37°C. After harvesting, the cells were incubated in PBS containing 0.5% BSA and 1 mM calcium with the different mAbs (10 μg/ml) for 30 minutes at 4°C and were washed with a 30-fold excess of ice-cold PBS/BSA. The cells were then incubated with PE-conjugated goat-anti-mouse-IgG for 30 minutes at 4°C and washed. The relative fluorescence intensity was measured by flow cytometry (FACScan, Becton Dickinson).

Statistics.
Student’s t-test for paired samples (two-tailed) was used for statistical analysis. Student’s t-test for independent samples was used where indicated.

RESULTS

Role of VE-cadherin in permeability and integrity of HBMEC monolayers.
In the initial series of experiments, the role of VE-cadherin in the control of HBMEC monolayer integrity was examined. Ab-mediated inhibition of VE-cadherin resulted in an increased permeability of HBMEC monolayers, whereas a non-blocking, isotype-matched VE-cadherin Ab or an irrelevant IgG1 did not have any effect on the permeability of HBMEC (Figure 1). Immunofluorescent staining of HBMEC showed a jagged distribution of VE-cadherin (Figure 2a) and colocalization with the ends of F-actin stress fibers (Figure 2c) [17,35]. Pretreatment of HBMEC monolayers with the blocking Ab against VE-cadherin resulted in a redistribution of VE-cadherin over the cell surface (Figure 2d) and a marked reorganization of the F-actin cytoskeleton (Figure 2e). This observation is in line with the increased HBMEC permeability being the result of loss of VE-cadherin-mediated cell-cell adhesion, indicating that VE-cadherin plays an important role in the regulation of the integrity of HBMEC monolayers.

Figure 1. VE-cadherin-mediated permeability of HBMEC monolayers. Cells were grown to confluency on FN-coated Transwell filters, prestimulated with IL-1β, followed by pretreatment for 30 minutes with Abs (10 μg/ml) and incubated for 3 hours with FITC-dextran 3000 in the upper compartment. Next, fluorescence in the lower compartment was measured in a fluorimeter (λex 485 nm; λem 525 nm) and expressed as percentage of basal fluorescence in that compartment. The cl75 Ab to VE-cadherin, but not its isotype 7H1, increased monolayer permeability significantly (*p<0.001). Data are means ± SEM of five independent experiments.
Figure 2. Inhibition of VE-cadherin alters VE-cadherin distribution and induces cytoskeletal reorganization. HBMEC were grown to confluence on FN-coated glass coverslips, pretreated with IL-1β, and VE-cadherin and F-actin were visualised by double immunofluorescence as described in Methods. The overlays show VE-cadherin in green (a) and F-actin in red (b); colocalization appears in yellow (c). Pretreatment with the c175 Ab (10 μg/ml) caused loss of junctional localization of VE-cadherin (d), and a reorganization of the actin cytoskeleton as revealed by the loss of stress fibers (e). As a result of these changes, colocalization was lost (f). Bar, 50 μm.

Figure 3. (a) Transmigration of primary CD34⁺ cells across FN. SDF-1α (30 ng/ml)-induced migration of CB CD34⁺ cells (hatched bars) across FN-coated filters was significantly higher than the migration of HL-60 cells (filled bars) or HV CD34⁺ cells (open bars) (*p<0.001). Data are means ± SD of four independent experiments. (b) SDF-1α-induced transendothelial migration of HL-60 cells. Dose-response of SDF-1α-induced migration of HL-60 cells across HBMEC shows a bell-shaped curve with an optimal migration at 70 ng/ml SDF-1α (filled bars). Open bars represent HBMEC that were pretreated for 30 minutes with Ab c175 to VE-cadherin (10 μg/ml), resulting in a significantly increased migration at 30 ng/ml SDF-1α (*p<0.05) and a shift of the dose for optimal migration from 70 to 30 ng/ml SDF-1α. Data are means ± SD of three independent experiments.
Role of VE-cadherin in transmigration of CD34$^+$ cells across HBMEC.

Purified CD34$^+$ cells from CB and HV and the human leukemic cell line HL-60, which all express CXCR-4, were tested for their ability to migrate across FN or HBMEC towards a gradient of SDF-1α. HL-60 cells showed a similar efficiency as did CD34$^+$ cells from HV in transmigration across FN to 70 ng/ml SDF-1α, whereas CB CD34$^+$ cells showed a higher migration efficiency (Figure 3a). However, CXCR-4 expression of HV and CB CD34$^+$ were similar (data not shown), indicating that differences in CXCR-4 expression could not explain the more efficient migration of CB CD34$^+$ cells. Because of the limited supply of primary CD34$^+$ cells, HL-60 cells were used as a model in dose-response studies of transmigration across HBMEC. The results show a bell-shaped response with optimal migration at 70 ng/ml SDF-1α (Figure 3b). When VE-cadherin was blocked on HBMEC, a shift of the optimal concentration from 70 to 30 ng/ml SDF-1α was observed (Figure 3b). Moreover, a significant increase in the transmigration of CD34$^+$ cells at 30 ng/ml SDF-1α was observed. This finding shows that VE-cadherin function is an important determinant of the efficiency of migration of CD34$^+$ cells across bone-marrow endothelium. Therefore, we analysed the VE-cadherin distribution during transmigration in more detail. We observed a focal loss of VE-cadherin as well as of β-catenin at sites of

![Figure 4](image-url)
transmigration of CD34⁺ cells (Figure 4a and data not shown). In addition, stress fibers seemed to converge at the periphery of the transmigrating CD34⁺ cell (Figure 4c). These observations suggest a co-ordinated interaction between the F-actin cytoskeleton and VE-cadherin at sites of CD34⁺ cell transmigration.

![Image of transmigration and stress fiber convergence](image.jpg)

Figure 5. Effect of inactivation of p21Rho on the transmigration of CD34⁺ cells and on the permeability and integrity of HBMEC monolayers. (a) HBMEC were treated for 6 hours with 10 μg/ml C3 transferase to inactivate p21Rho and were immunostained for F-actin and VE-cadherin. The treatment with C3 caused a loss of stress fibers (a), but staining of VE-cadherin remained localized to cell-cell junctions (b); colocalization appears in yellow (c). In addition to the C3 treatment, the monolayer was pretreated for 30 minutes with the c175 antibody (10 μg/ml), which caused a loss of HBMEC cell-cell adhesion (d) and diffuse staining of VE-cadherin over the surface (e). Bar, 50 μm. Inactivation of p21Rho had no effect on the SDF-1α-induced migration of CB CD34⁺ cells across HBMEC with or without 30 minutes pretreatment with the c175 antibody (10 μg/ml) (g). Inactivation of p21Rho also had no significant effect on the permeability to FITC-dextran 3000 of HBMEC (h). Data are means ± SD of three independent experiments.

Role of p21Rho in VE-cadherin-mediated transmigration of CD34⁺ cells and in the permeability and integrity of HBMEC monolayers.

Ab-mediated loss of VE-cadherin function was accompanied by a reorganization of the actin cytoskeleton (Figure 2). Together with the results shown in figure 4, these
Figure 6. Role for endothelial adhesion molecules in SDF-1α-induced transmigration of primary CD34⁺ cells across HBMEC. To determine the role of these adhesion molecules on CD34⁺ cell transmigration, HBMEC were cultured on Transwell filters and prestimulated as described under Methods. Prior to the addition of the CD34⁺ cells from HV or CB to the upper compartment, the monolayers on the filter were incubated for 30 minutes with blocking antibodies to adhesion molecules, followed by washing. (a) mAbs to ICAM-1 (10 μg/ml, α-ICAM-1) and to VCAM-1 (10 μg/ml, α-VCAM-1) inhibited the SDF-1α-induced migration of HV CD34⁺ cells. The combination of Abs to ICAM-1 and VCAM-1 (α-1+α-2) showed a significant inhibition of the transmigration (**p<0.01). Pretreatment of HBMEC with Ab c175 against VE-cadherin (10 μg/ml, filled bars) increased basal migration of HV CD34⁺ cells (**p<0.01). Under these conditions, ICAM-1 was still required for efficient migration. (**p<0.001). (b) mAbs to ICAM-1 (10 μg/ml) also inhibited the migration of CB CD34⁺ cells (**p<0.01). Similarly, mAbs VCAM-1 (10 μg/ml) inhibited the migration of CB CD34⁺ cells (∗p<0.05). In addition, the combination of mAbs to ICAM-1 and VCAM-1 (α-1+α-2) inhibited the transmigration (**p<0.01). Pretreatment of HBMEC with Ab c175 to VE-cadherin (10 μg/ml, filled bars) increased basal SDF-1α-induced migration of CB CD34⁺ cells, but mAbs to ICAM-1 were still able to inhibit the migration (**p<0.01). Data are means ± SD of at least three independent experiments.

Findings suggest that the actin cytoskeleton regulates VE-cadherin function in HBMEC. Regulation of endothelial contractility can be mediated by changes in cAMP levels [36] and by the small GTPase p21Rho [37,38]. p21Rho is required for actin stress fiber formation in response to extracellular stimuli in many cell types and has also been implicated in the organization of cadherin-based cell-cell adhesion in epithelial cells [39,40]. To investigate the role of p21Rho in control of transmigration of CD34⁺ cells across HBMEC and of HBMEC permeability, HBMEC were pretreated with C3 transferase. As a result, a loss of cytoplasmic F-actin staining (Figure 5a), but no change in VE-cadherin distribution (Figure 5b) was observed. When, in addition to C3 pretreatment, VE-cadherin was blocked, the protein became diffusely localized over the cell surface (Figure 5d and e).
Inactivation of p21Rho by C3 did not significantly affect the transmigration of CD34\(^+\) cells across HBMEC (Figure 5g), indicating a minor role for p21Rho in the control of the transmigration of CD34\(^+\) cells. Moreover, C3 pretreatment also did not affect the permeability of HBMEC (Figure 5h).

![Crosslinked](image)

**Figure 7. Effect of crosslinking of adhesion molecules on the permeability of HBMEC monolayers.** The HBMEC monolayer permeability to FITC-dextran 3000 was measured following pretreatment of the endothelial cells with mAbs to ICAM-1, VCAM-1, PECAM-1 or IgG1 (1 hour, 10 \(\mu\)g/ml). Thirty minutes prior to the addition of FITC-dextran 3000, the antibodies were crosslinked with goat-anti-mouse-IgG. Fluorescence in the lower compartment was measured after 3 hours. Crosslinking of ICAM-1 decreased the permeability of HBMEC (p<0.001, compared to control), whereas crosslinking of VCAM-1 showed an increase in permeability HBMEC (p<0.001, compared to control). Also PECAM-1/CD31 induced permeability after crosslinking HBMEC (p<0.001, compared to control). Data are means ± SD of at least 4 independent experiments.

**Role of ICAM-1 and VCAM-1 in VE-cadherin-mediated transmigration of CD34\(^+\) cells.**

The VLA-4 and VLA-5 integrins are necessary for efficient migration of CD34\(^+\) cells across HBMEC \([2,11,41,42]\). Therefore, we investigated which adhesion molecules that are known ligands for VLA-4 and VLA-5 mediate the transmigration of CD34\(^+\) cells across HBMEC. ICAM-1 is highly expressed on IL-1\(\beta\)-prestimulated HBMEC, whereas VCAM-1 is expressed at a lower level (data not shown). Pretreatment of the HBMEC monolayers with blocking Abs to ICAM-1 or VCAM-1 inhibited the transmigration of HV and CB CD34\(^+\) cells across HBMEC. The combination of Abs to ICAM-1 and VCAM-1 inhibited the transmigration for HV and CB CD34\(^+\) cells significantly (Figure 6a and b). Pretreatment of HBMEC with Abs to PECAM-1 did not affect the transmigration of both HV and CB CD34\(^+\) cells (data not shown). Following pretreatment of the HBMEC with mAbs to VE-cadherin, which increased the transmigration of CD34\(^+\)
cells across HBMEC for both cell types, the transmigration was still dependent on ICAM-1 (Figure 6a and b) and VCAM-1 (data not shown).

Figure 8. Effect of crosslinking of adhesion molecules on the integrity of HBMEC monolayers. HBMEC were grown to confluency on FN-coated glass coverslips and stained for VE-cadherin and F-actin. The images show F-actin in red (a,d,g) and VE-cadherin in green, stained with a polyclonal Ab (b,e,h); colocalization appears in yellow (c,f,i). HBMEC monolayers were incubated for 30 minutes with mAbs to ICAM-1, followed by 30 minutes of crosslinking with goat-anti-mouse-IgG. F-actin staining showed induction of stress fibers, but this was not accompanied by the formation of discernible gaps (d). VE-cadherin was localized normally to cell-cell junctions (e). Crosslinking of VCAM-1 induced stress fibers and gaps were formed in the HBMEC monolayer (g), with diffuse VE-cadherin localization at sites of gap formation (h). Staining of endothelial nuclei was due to aspecific binding of the ALEXA 488-labeled goat-anti-rabbit-Ig secondary antibody. Bar, 20 μm.

Role of ICAM-1 and VCAM-1 in permeability and integrity of HBMEC monolayers.

As was shown in figure 1, loss of VE-cadherin function increased the permeability of HBMEC monolayers. To establish the effects of signaling induced by specific adhesion molecules on VE-cadherin-mediated cell-cell adhesion and thus on monolayer integrity, permeability assays in combination with crosslinking were performed. Crosslinking of ICAM-1 with specific mAbs resulted in a small, but significant decrease in the HBMEC permeability (Figure 7). In contrast, crosslinking of VCAM-1 or PECAM-1 induced a significant increase in the permeability of HBMEC monolayers (Figure 7). These results imply that ICAM-1 plays no direct role in reducing endothelial cell-cell contact, whereas VCAM-1 and PECAM-1 may promote signaling which controls VE-cadherin. Analysis of the F-
VE-cadherin modulates migration of HPC

actin cytoskeleton showed that crosslinking of ICAM-1 induced stress fiber formation (Figure 8d), but this was not accompanied by discernible gaps in the HBMEC monolayer (Figure 8e). VE-cadherin at cell-cell junctions was distributed normally (Figure 8f). In contrast, crosslinking of VCAM-1 did induce gaps in between endothelial cells (Figure 8g) and focal loss of VE-cadherin at sites of gap formation was observed (Figure 8h). Similar results were obtained with crosslinking of PECAM-1 (data not shown).

DISCUSSION

The present study demonstrates that VE-cadherin is a crucial regulator of the permeability of human bone-marrow endothelial cells and that its adhesive properties control the efficiency of migration of hematopoietic progenitor cells (CD34+ cells) across HBMEC. In addition, endothelial adhesion molecules such as ICAM-1 and VCAM-1 were found to be important for CD34+ cell adhesion and for the induction of endothelial signaling, regulating VE-cadherin function and HBMEC monolayer integrity.

Transmigrating CD34+ cells induced a focal loss of VE-cadherin and of β-catenin at endothelial cell-cell junctions. This focal loss of VE-cadherin was reversible; the normal, jagged pattern of VE-cadherin distribution was restored following the transmigration assays. A similar phenomenon was also reported by Allport et al. [35], who studied monocyte migration across HUVEC under flow, albeit in the absence of a chemotactic gradient. One of the proposed mechanisms for the loss of VE-cadherin function during leukocyte passage is the trapdoor mechanism: the VE-cadherin-catenin complex is mechanically pushed aside by the migrating leukocyte and simply re-adheres after leukocyte passage [35]. However, despite the fact that the VE-cadherin distribution was lost at sites of CD34+ cell transmigration, the migration across HBMEC remained dependent on ICAM-1 and VCAM-1-mediated adhesion in the absence of VE-cadherin function.

Conflicting results on the role of ICAM-1 and VCAM-1 in transmigration of CD34+ cells have been published [43,44]. We therefore tested the hypothesis that leukocyte-endothelium interactions, mediated by ICAM-1 and VCAM-1, induce endothelial signaling that controls cell-cell adhesion, i.e. VE-cadherin function. Crosslinking experiments supported a role for VCAM-1 in the regulation of endothelial cell-cell junctions. In contrast, ICAM-1 failed to induce gaps between the cells after crosslinking, despite the induction of actin stress fibers. This suggests that ICAM-1 is important for strong adhesion of CD34+ cells to the
HBMEC and that VCAM-1, besides its role in CD34⁻ cell-adhesion, induces signaling to control HBMEC cell-cell contacts. Several reports have suggested a signaling role for ICAM-1 during leukocyte transmigration, e.g. through activation of p21Rho [36,45,46]. Our crosslinking studies indicate that also in HBMEC, ICAM-1 may activate Rho, as deduced from the induction of stress fibers. However, this response was not sufficient to induce loss of HBMEC integrity. In line with this, inhibition of p21Rho by the C3 transferase did not affect the transmigration of CD34⁻ cells or the integrity of HBMEC monolayers, suggesting only a minor role for the Rho GTPase in the control of CD34⁻ cell transmigration. These findings suggest differential, possibly cell-type specific use of a Rho-signaling pathway in the control of endothelial monolayer integrity during leukocyte transmigration.

Based on these data, we suggest that homing of CD34⁻ cells requires ICAM-1 for firm adhesion to the bone-marrow endothelium and that VCAM-1 may be required to induce focal loss of VE-cadherin function via as yet uncharacterised signal transduction pathways. It has been reported that loss of VE-cadherin function promotes increased neutrophil recruitment in a murine model of thioglycollate-induced acute peritonitis and increases vascular permeability in the heart and lungs of mice [16,47]. Together with these published data, our current results suggest that adhesion molecule-mediated regulation of VE-cadherin function in human bone-marrow endothelial cells is essential for efficient transendothelial migration and thus plays an important role in the homing of CD34⁻ cells.

ACKNOWLEDGMENTS

We thank D.Roos for critically reading the manuscript. JDvB and ECA are supported by a grant from the Dutch Cancer Foundation (grant no. CLB99-2000).

REFERENCES

VE-cadherin modulates migration of HPC

new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. J. Exp. Med. 185:111-120.


VE-cadherin modulates migration of HPC


