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Intercellular Adhesion Molecule (ICAM)-1, But Not ICAM-2, Activates RhoA and Stimulates c-fos and rhoA Transcription in Endothelial Cells

Paul W. Thompson,*† Anna M. Randi,‡ and Anne J. Ridley2*†

ICAM-1 and -2 are integrin-binding Ig superfamily adhesion molecules that are important for leukocyte transmigration across endothelial monolayers. ICAM-1 cross-linking is known to activate the small GTPase RhoA and induce stress fiber formation in endothelial cells, but ICAM-2 signaling has not been investigated. In this study, we compare ICAM-1 and ICAM-2 signaling and localization in HUVECs. Although ICAM-1 and ICAM-2 both localize with the actin-binding protein moesin in apical microvilli, only ICAM-1 colocalizes with moesin after cross-linking. Unlike ICAM-1, ICAM-2 does not activate RhoA or alter actin cytoskeletal organization. Interestingly, ICAM-1 stimulates transcription of c-fos, a known early response gene. In addition, it up-regulates rhoA expression, suggesting that it activates a positive feedback pathway after RhoA activation. These results indicate that in endothelial cells, ICAM-1, but not ICAM-2, rapidly stimulates signaling responses involving RhoA.

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the actin cytoskeleton (21), and play an important role in lamellipodium extension (22), cell–cell adhesion (23), microvillus assembly, and cytokinesis (23). Ab-cross-linked ICAM-1 coclusters with ERM proteins in endothelial cells (17). The N-terminal domain of ERM proteins (N-ERMAD) can interact with both phosphoinositides and a number of proteins, while the C-terminal domain of ERM proteins binds to filamentous actin (F-actin). There is evidence that Rho activation occurs downstream of ERMs as N-ERMADs can interact with RhoGDI, which normally holds Rho in an inactive GDP-bound complex in the cytoplasm. N-ERMAD binding to RhoGDI facilitates exchange of GDP for GTP on Rho, presumably because Rho is released from RhoGDI (24). Also, more recently, the ICAM-1 cytosolic tail has been shown to interact with the Src homology 2 domain containing tyrosine phosphatase-2 (SHP-2) in a phosphotyrosine-dependent manner (25).

In this study, we compare the effects of ICAM-1 and -2 cross-linking in primary human umbilical cord endothelial cells upon cytoskeletal arrangements and gene expression. We show that although ICAM-1, ICAM-2, and moesin all localize in microvilli in unstimulated cells, only ICAM-1 cross-linking coclusters moesin, activates RhoA, and causes stress fiber formation, whereas ICAM-2 cross-linking does not affect the actin cytoskeleton. Furthermore, ICAM-1 cross-linking induces rhoA and c-fos gene expression. Thus, we provide a mechanistic basis for the difference between the roles of ICAM-1 and -2 in leukocyte transmigration.

Materials and Methods

Materials

Reagents were obtained from the following sources: medium 199 modified Earle’s salt solution and HBSS (Life Technologies, Paisley, U.K.); Nutridoma NS (Boehringer Mannheim, Lewes, U.K.); pooled HUVECs and endothelial growth medium (EGM-2) (Clonetics, San Diego, CA); human fibronectin, heparin, endothelial cell growth supplement, tetramethylrhodamine isothiocyanate (TRITC)-phallolidin, HEPES, collagen type IV, aprotinin, leupeptin, and other lysis buffer components, unless otherwise stated (Sigma-Aldrich, Gillingham, U.K.); TNF-α (Insight Biotechnologie, Wembley, U.K.); mouse monoclonal anti-ICAM-1 Ab (clone BBIG-I1; R&D Systems, Abingdon, U.K.); mouse monoclonal anti-ICAM-2 Ab (clone CBR-IC2/2; Alexis Biochemicals, Nottingham, U.K.); goat polyclonal anti-CPERM Ab (C-15; Sigma-Aldrich); Alexa 488-labeled goat anti-mouse IgG and Alexa 546-labeled goat anti-mouse IgGAb (Molecular Probes, Leiden, The Netherlands); FITC- and TRITC-labeled goat anti-mouse and goat anti-rabbit Abs (Southern Biotechnology Associates, Birmingham, AL); Cy5-conjugated goat anti-rat Ab (Jackson ImmunoResearch Laboratories, West Grove, PA); rabbit polyclonal anti-myosin II Ab (Biogenesis, Poole, U.K.); mouse monoclonal anti-RhoA Ab (Santa Cruz Biotechnology, Santa Cruz, CA); HRP-conjugated goat anti-mouse Ab (Bio-Rad, Hemel Hempstead, U.K.); and Fugene 6 (Boehringer Mannheim). Rabbit polyclonal anti-moesin Ab was generously provided by Dr. P. Mangeat (Université Montpellier II, Montpellier, France); rat monoclonal anti-COOH (Thr-558) C-terminally phosphorylated ERM proteins (anti-CPERM, 2975) were generously provided by Dr. S. Tsukita (Kyoto University, Kyoto, Japan). Integrin peptide P6 was generously provided by Dr. S. Hart (Institute of Child Health, London, U.K.). Lightcycler apparatus and PCR reagents were obtained from Roche (Basel, Switzerland), primers for rhoA gene expression analysis were obtained from Life Technologies, and primers for c-fos gene expression analysis were obtained from Sigma-Genosys (Pampsisford, U.K.).

Cell culture

HUVECs were isolated from umbilical cords using 0.1% collagenase type II. The cells were cultured in TC Nunclon flasks in medium 199 modified Earle’s salt solution containing 1.25 g/L NaHCO3 and Glutamax and supplemented with 20% FCS, 100 μg/ml endothelial cell growth supplement, 1% Nutridoma NS, and 100 μg/ml heparin. Alternatively, HUVECs from Clonetics were grown in EGM-2. Cells were cultured at 37°C in humidified air containing 5% CO2. For experiments, cells were used between one and three passages.

For immunofluorescence experiments, cells were grown on glass coverslips coated with 10 μg/ml human fibronectin until confluent. For biochemical experiments, cells were grown on 6- and 10-cm dishes coated with human fibronectin until confluent. To obtain quiescent-starved cells, the culture medium was replaced by medium containing 10% FCS, but no heparin or other growth factors. Cells were incubated in this medium for no longer than 24 h. Cells grown in EGM-2 were starved in 1% FCS. No differences between HUVEC responses were observed between cells grown in different media.

Receptor clustering and immunofluorescence

To induce receptor clustering, either mouse monoclonal anti-ICAM-2 Ab was added to starved cells at a final concentration of 10 μg/ml for 60 min, or mouse monoclonal anti-ICAM-1/ICAM-2 were added to cells that had been stimulated for 24 h with 10 ng/ml TNF-α. After incubation with primary Abs, TNF-α and the primary Abs were removed from the cell medium and 10 μg/ml of Alexa 488-labeled goat anti-mouse Ab was added to the cells for between 15 and 60 min. Cells were then washed three times in TBS (10 mM Tris, pH 7.5, 150 mM NaCl) containing 0.25% BSA, fixed in 4% paraformaldehyde for 20 min at room temperature, permeabilized for 6 min with 0.2% Triton X-100, and then incubated with 1 μg/ml TRITC-phallolidin for 45 min to stain actin filaments, or for 45 min with rabbit polyclonal anti-moiesin Ab diluted 1/200, or rabbit polyclonal anti-myosin II Ab diluted 1/25, followed by the appropriate TRITC-labeled secondary Ab. The specimens were mounted in moviol. To observe responses in cells without receptor clustering, cells were incubated with primary Abs as before for 60 min. These cells were then washed, fixed, and stained with the secondary Ab for 45 min. Staining for intracellular epitopes was then conducted as described above. All incubations were conducted in TBS containing 0.25% BSA.

To use the phosphospecific anti-CPERM Ab, a different fixation procedure was used as previously described (38). In brief, after receptor clustering, cells were fixed in 10% TCA in distilled water at 4°C for 20 min. All further staining was conducted in TBS containing 0.25% BSA. Cells were incubated with rat monoclonal anti-CPERM Ab (2975) for 45 min, followed by Cy5-conjugated goat anti-rat Ab for 45 min.

Confocal laser scanning microscopy was conducted with an LSM 510 (Zeiss, Welwyn Garden City, U.K.) mounted over an affinity corrected Axioplan microscope (Zeiss). Image files were collected as a matrix of 1024 × 1024 pixels describing the average of eight frames scanned at 0.062 Hz where FITC, TRITC, and Cy5 were excited at 488, 545, and 633 nm and visualized with 540 ± 25, 608 ± 22, and 690 ± 30 nm bandpass filters, respectively, where the levels of interchannel cross-talk were insignificant.

DNA constructs and transfection of HUVECs

pCDM8-ICAM-1 (human) has been previously described (26). The cDNA encoding human ICAM-2 (3) was subcloned into pCDNA3 using EcoRl and NotI restriction sites. HUVECs were transfected using a mixture of P6 integrin peptide and Lipofectin (Life Technologies) as previously described (27). Briefly, P6 integrin-targeting peptide, lipofectin, and plasmid DNA were allowed to form a complex, which was incubated with subconfluent cells in OptiMEM for 4 h. Cells were then washed in OptiMEM and grown to confluence in their normal growth medium (medium 199 with supplements).

RhoA activity assay

GST-rhotekin Rho-binding domain (TRBD; a gift from Dr. M. Schwartz, Scripps Institute, San Diego, CA) was expressed in E. coli. GST-rhotekin TRBD was expressed in E. coli. GST-rhotekin TRBD was purified as previously described (28). TRBD kinase assays were performed on GST-TRBD beads (50 μg) and 100 μg of GST-TRBD beads (50 μg) were incubated with 1 μg/mg GLP-1 and pGlu-GLP-1 (10-cm dishes) were subject to Ab-induced ICAM-1/ICAM-2 clustering as described above. Control dishes were treated with primary or secondary Abs alone, or were given fresh medium before lysis. Cells were lysed in lysis buffer (50 mM Tris, pH 7.3, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 500 mM NaCl, 10 mM MgCl2, 10 μg/ml each of leupeptin and aprotinin, 1 mM PMSF). Lysates were incubated with 20 μg of GST-TRBD beads for 1 h on ice. Beads were washed in buffer B (50 mM Tris pH 7.3, 1% Triton X-100, 150 mM NaCl, 10 mM MgCl2, 10 μg/ml each of aprotinin and leupeptin, 100 μM PMSF). Proteins retained on the GST-TRBD beads were resolved by 13% SDS-PAGE. Bound Rho A was detected by Western blotting using mouse monoclonal anti-RhoA Ab. Quantification of relative RhoA activity was performed using the photo- densitometer program Quantity One (Bio-Rad). The amount of activated RhoA was normalized to the amount of total RhoA in the corresponding lysate for each treatment. The significance of any difference in RhoA activity after ICAM clustering was determined by Student’s t test.
Gene expression analysis

TNF-α-activated HUVECs (10 ng/ml, 24 h) were stimulated with either ICAM-1 or -2 cross-linking Abs for either 60 or 90 min. Total RNA was isolated using the Qiagen RNeasy kit (Valencia, CA). First strand-cDNA synthesis was performed on the total RNA preparations to provide a cDNA template for gene expression analysis.

Qualitative gene expression was performed using the Lightcycler system. Primers for real-time PCR were designed close to the 3′ end of rhoA and c-fos and were as follows: rhoA 5′-ATGTGCCACAGGTGGTTGA GAAC; rhoA 3′-CGTGGGACAGAAATGCCTTGCT; c-fos 5′-TCAC CTCGCCCTTCCTCTCAAT; c-fos 3′-GCTGCATAGAAGGACCAGATAG.

Standard curves were generated using rhoA cDNA as a template and the rhoA primers. Therefore, samples were analyzed for gene copy numbers as compared with the rhoA standard curve. In the case of samples analyzed for c-fos, the standard curve was still made from rhoA transcripts so this analysis could only be qualitative. Normalization of the samples was conducted by assessing the GAPDH mRNA content in each sample against a GAPDH standard curve. For qualitative analysis, all 0-min timepoint samples were taken as the reference point, with changes induced by stimulation represented as changes relative to the 0-min timepoint. Results were obtained from two separate stimulations.

RhoA protein levels were also determined after ICAM-1 and -2 cross-linking. After cross-linking, cells were lysed in 2X Laemmli sample buffer. Proteins were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membrane followed by immunoblotting for RhoA. Blots shown are representative of three separate experiments.

Results

Both ICAM-1 and -2 colocalize with moesin and F-actin in HUVECs

ICAMs have been shown to interact with ERM proteins in vitro and using exogenously expressed proteins. Endothelial cells express ICAM-2 constitutively and can be induced to express high levels of ICAM-1 by inflammatory stimuli. Therefore, immunofluorescence techniques were used to investigate the subcellular localization of ICAMs and ERM proteins in primary endothelial cells. In resting HUVECs, ICAM-2 colocalized with moesin at intercellular junctions and at the apical surface (Fig. 1, a–c). Interestingly, colocalization also occurred in distinct microvillus-like structures on the apical surface. These structures also contained F-actin (Fig. 1, d–f) and are therefore likely to be microvilli. Colocalization of moesin and ICAM-2 was also observed in TNF-α-activated HUVECs (data not shown). ICAM-1 was not expressed significantly in resting, unstimulated HUVECs (data not shown; Ref. 1). After 24 h of TNF-α stimulation (10 ng/ml), ICAM-1 expression was highly induced and colocalized with moesin at intercellular junctions and in microvillus-like structures with moesin (Fig. 1, g–i), similar to the distribution of ICAM-2. These results indicate that leukocyte-binding receptors ICAM-1 and -2 are optimally positioned on endothelial cell microvilli for interaction with β2 integrins.

ICAM-1, but not ICAM-2, cross-linking induces stress fiber formation and moesin coclustering

It has previously been shown that Ab-induced ICAM-1 clustering induces the activation of Rho and the formation of stress fibers in rat brain-derived microvascular endothelial cell lines (13). Therefore, we investigated the effects of ICAM-1 and -2 Ab-induced

FIGURE 1. ICAMs colocalize with moesin in endothelial cell microvilli. Confluent HUVECs were starved for 24 h in medium 199 containing 10% FCS. Starved monolayers were either untreated (a–f) or treated with 10 ng/ml TNF-α for 24 h (g–i), fixed in 4% paraformaldehyde, and stained for ICAM-2 (a, c, d, and f) or ICAM-1 (g and i) with mouse mAbs. Cells were then refixed, permeabilized in 0.2% Triton X-100, and stained for moesin (b, c, h, and i) using a rabbit polyclonal Ab, and for F-actin (e and f) using TRITC-phalloidin. In merged images, ICAMs are shown in green, while F-actin and moesin are shown in red. Bars, 10 μm.

FIGURE 2. ICAM-1 cross-linking induces actin stress fiber formation in HUVECs. Starved monolayers were either treated with 10 ng/ml TNF-α for 24 h (a–f) or left untreated (g and h). Untreated cells (g and h) had been transfected 5 days previously with pCDS8-ICAM-1(human). Cells were incubated with 10 μg/ml mouse monoclonal anti-ICAM-1 Ab for 60 min. Cells were either washed, fixed, and stained with Alexa 488-goat anti-mouse Ab for 60 min (a and b), or washed in conditioned medium and then incubated with the secondary Ab for 30 min (e and f) or 60 min (c–e and h). All cells were then fixed, permeabilized, and stained for either F-actin (b, d, and h) using TRITC-phalloidin, or moesin (f) using a rabbit polyclonal Ab. Bars, 10 μm.
cross-linking on the actin cytoskeleton of HUVECs. Ab-cross-linking of ICAM-1 for 60 min on HUVECs induced the formation of F-actin bundles which aligned parallel to the elongated axis of the cell (compare Fig. 2, b and d). ICAM-1 cross-linking also induced the assembly of myosin II-containing filaments (data not shown), confirming that the newly formed F-actin structures were stress fibers, and this was accompanied by the appearance of intercellular gaps, indicative of increased contractility.

Ab-induced cross-linking led to the accumulation of large ICAM-1 clusters (Fig. 2c), as previously described (17). The majority of these large ICAM-1 clusters were on the cell surface and not internalized (data not shown). As we showed that ICAM-1 colocalized with moesin in microvilli (Fig. 1), we investigated the effects of Ab-induced ICAM-1 cross-linking on the localization of moesin. ICAM-1 cross-linking caused a dramatic redistribution of moesin, so that moesin colocalized with ICAM-1 clusters (Fig. 2, e and f), consistent with our previous report of ERM colocalization with cross-linked ICAM-1 (17).

To determine whether TNF-α signaling is required for ICAM-1 to induce stress fiber assembly, we transfected HUVECs with exogenous ICAM-1 so that they could be cross-linked on resting, unstimulated cells. Under these conditions, ICAM-1 cross-linking was able to stimulate the formation of stress fibers (Fig. 2, g and h), indicating that TNF-α prestimulation is not required for this response.

In contrast to ICAM-1, Ab cross-linking of ICAM-2 for 60 min on resting HUVECs only induced the formation of small clusters of ICAM-2 and had no effect on the arrangement of F-actin (Fig. 3, a–d). Myosin II staining also confirmed that no increase in stress fibers occurred in response to ICAM-2 cross-linking (data not shown). TNF-α treatment for 24 h induced a small increase in stress fibers relative to quiescent HUVECs (compare Fig. 3, b and f), but ICAM-2 cross-linking after TNF-α stimulation had no effect upon the number or organization of F-actin cables (Fig. 3, e–h). Surprisingly, moesin did not cocluster with ICAM-2 (Fig. 3, i and j), although it colocalized with moesin in microvilli before clustering (Fig. 1, a–c).

**ICAM-2 does not activate RhoA**

As stress fiber formation is dependent on RhoA (15), we assayed the effects of ICAM-1 and -2 cross-linking upon RhoA activity using the TRBD to affinity-precipitate endogenous GTP-bound RhoA from endothelial cell lysates (28). RhoA was activated between 15 and 30 min after ICAM-1 clustering and remained activated at 1 h (Fig. 4). RhoA activity showed a 2.5-fold increase as compared with resting levels at this time point. ICAM-2 clustering, however, had no effect on RhoA activity. This data confirms results from immunofluorescence experiments in Figs. 2 and 3, and

![Figure 3](image3.png)

**FIGURE 3.** ICAM-2 cross-linking does not induce actin cytoskeleton rearrangement. Starved monolayers were either left untreated (a–d, i, and j) or treated with 10 ng/ml TNF-α for 24 h (e–h). Cells were incubated with 10 μg/ml mouse monoclonal anti-ICAM-2 for 60 min. Cells were either washed, fixed, and stained with Alexa 488-goat anti-mouse Ab (a, b, e, and f), or washed in conditioned medium and then incubated with the secondary Ab for 60 min (c, d, and g–j). All cells were then fixed, permeabilized, and stained for either F-actin (b, d, f, and h) using TRITC-phalloidin, or moesin (i and j) using a rabbit polyclonal Ab. Bar, 10 μm.

![Figure 4](image4.png)

**FIGURE 4.** ICAM-1, but not ICAM-2, cross-linking induces RhoA activation. a, Rho activity was assayed using GST-TRBD to pull-down GTP-bound Rho only. Confluent 10-cm dishes of starved HUVECs were treated with TNF-α (10 ng/ml) for 24 h. Following ICAM-1 or -2 cross-linking, cells were lysed at the indicated timepoints. Lysates were then incubated with GST-TRBD on glutathione beads for 60 min and bound proteins were resolved by 13% SDS-PAGE (upper panel). Proteins were transferred to polyvinylidene difluoride membranes and immunoblotted for RhoA using a mouse monoclonal anti-RhoA Ab. A fraction of each lysate was retained to determine the relative level of Rho (total Rho) in each lysate by immunoblot analysis (lower panel). 1’, primary mouse anti-ICAM-1 or -2 Ab alone; 2’: secondary goat anti-mouse Ab alone. Blots shown are representative of three separate experiments. b, Relative RhoA activation was quantified using photodensitometry. The data represent means ± SD of three independent experiments. Densitometry readings for activation were normalized to “Total Rho” densitometry readings for each individual treatment. GAM: secondary goat anti-mouse Ab alone; MoMo: primary mouse anti-ICAM-1 or -2 Ab alone. RhoA activation induced by ICAM-1 clustering was significantly different from that produced by ICAM-2 clustering (p < 0.005) as determined by the paired Student’s t test.
indicates that the effects of ICAM-1 and -2 cross-linking on the endothelial cytoskeleton are dramatically different.

**ICAM-1 cross-linking induces rhoA and c-fos gene expression**

RhoA has been shown to stimulate transcription of the c-fos gene through activation of the SRE (29). As ICAM-1 cross-linking activated RhoA, we tested whether c-fos expression was affected by ICAM-1 or -2 cross-linking. ICAM-1 cross-linking induced a dramatic 6-fold increase in c-fos mRNA levels (Fig. 5a), returning to resting levels after 90 min of ICAM-1 cross-linking. In contrast, 60 min of ICAM-2 cross-linking induced a relatively small 1.4-fold increase in c-fos mRNA levels. It is possible that this small increase was caused by addition of Abs alone, rather than a specific consequence of ICAM-2-induced signaling pathways.

Interestingly, ICAM-1 cross-linking also induced a small increase in rhoA mRNA expression levels (Fig. 5b). At 90 min after ICAM-1 cross-linking, a 1.5-fold increase in rhoA mRNA levels was observed as compared with resting levels. This increase in mRNA was paralleled by an increase in RhoA protein levels after ICAM-1 cross-linking (Fig. 5c). Quantification of the increase in protein levels by photodensitometry showed that at 135 min of ICAM-1 cross-linking, the level of RhoA protein was increased by 1.5-fold compared with resting levels. In contrast, ICAM-2 cross-linking did not induce an increase in rhoA mRNA or protein levels. In fact, at 90 min of ICAM-2 cross-linking, there was a small decrease in rhoA mRNA levels (Fig. 5b). There results suggest that activation of RhoA may feed back to stimulate an increase in RhoA protein levels.

**Discussion**

ICAM-1 and ICAM-2 are related leukocyte-binding receptors on endothelial cells involved in mediating leukocyte transmigration across the endothelium. As leukocyte-endothelial interaction can trigger responses in endothelial cells (13, 17), we have used Ab-induced cross-linking to compare responses induced by ICAM-1 and -2. We have shown that ICAM-1 and -2 both colocalize with the ERM family protein moesin and F-actin in endothelial cell microvilli. However, the responses to ICAM-1 and ICAM-2 are strikingly different. Ab cross-linked ICAM-1 coclusters moesin, activates RhoA to induce the formation of stress fibers in HUVECs, and induces expression of rhoA and c-fos genes, whereas cross-linked ICAM-2 does not colocalize with moesin or alter RhoA activity.

**ICAM localization and ERM proteins**

We have shown that both ICAM-2 in resting cells and ICAM-1 on activated cells localize with moesin in apical microvilli. This is consistent with previous reports showing the presence of CD44 and exogenously expressed ICAM-2 at microvilli in fibroblasts, although fibroblasts do not express endogenous ICAM-2. Positioning of leukocyte-binding receptors on endothelial cell microvilli is likely to facilitate leukocyte capture. We have observed a high level of C-terminal phosphorylation of ERM proteins in HUVECs (data not shown), indicating that they are predominantly in an active conformation and are therefore likely to link ICAMs to F-actin at microvilli.

**ICAM-1 and RhoA activation**

We have found that ICAM-1 cross-linking on primary endothelial cells induces the formation of stress fibers and the activation of RhoA, in accordance with previous results with a rat brain-derived endothelial cell line (12). Although ICAM-1 is only normally expressed in HUVECs following activation with cytokines such as TNF-α, cytokine stimulation is not required for ICAM-1 responses as cross-linking of exogenously expressed ICAM-1 on unstimulated HUVECs can still induce stress fiber formation. The level of ICAM-1 engaged by Ab in transfected cells is considerably lower than that on TNF-α-stimulated cells, and may be similar to the amount of ICAM-1 normally engaged by monocyte binding.

In contrast to ICAM-1, ICAM-2 does not activate RhoA, either in resting HUVECs or in TNF-α-stimulated HUVECs. Ab-induced ICAM-2 clusters are very small and do not aggregate into larger clusters unlike those formed by ICAM-1. Both ICAMs can bind to
the same ligand, the β₂ integrin, LFA-1, but their short cytoplasmic tails are markedly different in sequence and may therefore interact with different signaling proteins (1, 3, 30). Several proteins have been reported to interact with the ICAM-1 cytosolic tail (20, 25, 31), and of these, the SHP-2 and ERM proteins could be involved in ICAM-1 signal transduction. SHP-2 has recently been shown to interact with a poorly conserved immunoreceptor tyrosine-based inhibitory motif contained within the cytosolic tail of ICAM-1, after ICAM-1-mediated adhesion of cells to fibrinogen (25). SHP-2 has been reported to down-regulate RhoA activity in fibroblasts and epithelial cells (32, 33). ICAM-2 would not be expected to bind to SHP-2 as the sequence in the ICAM-1 tail that binds SHP-2 is not conserved in ICAM-2. Alternatively, ERM proteins could mediate RhoA activation downstream of ICAM-1 (34). Rho-GDI has been shown to interact with moesin, and this interaction with ERM proteins causes a decrease in the ability of Rho-GDI to inhibit nucleotide exchange on Rho (24), leading to Rho activation. Results from this study show that moesin coclusters with ICAM-1 rather than ICAM-2 and therefore it is possible that an ICAM-1/moesin interaction is required for signaling to RhoA. However, it is also likely that the function of ERM interaction with ICAMs is to stabilize interaction with the actin cytoskeleton.

ICAM-1 induces gene expression

In this study, we show that ICAM-1 cross-linking in TNF-α-activated HUVECs stimulates expression of c-fos and rhoA. ICAM-1 cross-linking has previously been reported to stimulate expression of the leukocyte adhesion molecule VCAM-1 and to activate the transcription factor activator protein (AP)-1 in resting endothelial cells (35), but ICAM-1-mediated changes in transcription in TNF-α-stimulated cells, which are more physiologically relevant as they express high levels of ICAM-1, have not been investigated before. Active RhoA has been shown to induce gene expression through activation of the SRE (29). In particular, RhoA can enhance transcriptional activation of c-fos, egr-1, and cox-2. Therefore, the ICAM-1-induced stimulation of c-fos transcription correlates well with the observed activation of RhoA. It is extremely interesting that ICAM-1 cross-linking induced up-regulation of both rhoA mRNA and RhoA protein levels. This may constitute a novel positive feedback response to RhoA activation, providing more RhoA for subsequent activation in response to further leukocyte binding. Little is known about how rhoA gene expression is regulated, although recently it was found that inhibition of the Ets family transcription factor Erg led to a decrease in rhol expression in endothelial cells (36). Therefore, it would be interesting to investigate whether Erg contributes to the observed up-regulation of rhol mRNA in response to ICAM-1.

Differences in ICAM-1 and -2 signaling and their biological roles

ICAM-1 is well-characterized for its role in endothelial cells during inflammation, whereas endothelial ICAM-2 does not appear to be important in inflammation, and in fact, proinflammatory cytokine stimulation of HUVECs down-regulates ICAM-2 protein levels (6). ICAM-2 can, however, contribute to monocyte transmigration through unstimulated endothelial cell monolayers (8), and transmigration of different T cell populations across ICAM-1-deficient endothelium (9). In the light of this evidence, and because ICAM-2 binds to the leukocyte integrin LFA-1, it had been proposed that ICAM-2 is involved in leukocyte recirculation. Recently, however, ICAM-2 was shown to mediate DC trafficking through its interaction with the C-type lectin, DC-specific ICAM-grabbing nonintegrin (5). DCs are professional APCs that continuously survey the tissues for incoming foreign Ags. Immature DCs have to migrate from the blood into the periphery and this is not dependent upon inflammatory signals. Therefore, constitutive expression of ICAM-2 on endothelial cells may be important for the transmigration of immature DCs.

Our results showing that ICAM-1, but not ICAM-2, activates RhoA, induces actin reorganization, and stimulates gene expression fit with their different roles in leukocyte transmigration. Under inflammatory conditions, where ICAM-1 is involved, RhoA activation would contribute to the inflammatory response of endothelial cells through its effects in disrupting intercellular junctions, enhancing endothelial permeability (16, 37). Increased transcription of selected genes is likely to contribute to the progression and eventual resolution of the endothelial response to inflammation. During routine leukocyte trafficking, such as immature DC transmigration, these responses to RhoA would be detrimental as they would promote vascular leakage, and thus it makes sense that ICAM-2 does not activate Rho. Indeed, it may actively inhibit Rho signaling, as indicated by the decrease in rhol mRNA levels following ICAM-2 cross-linking. Therefore, the role of endothelial cell signaling in leukocyte transmigration is likely to be very different in inflammatory compared with noninflammatory conditions.

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References


