Decrease in the Amount of Focal Adhesion Kinase (p125FAK) in Interleukin-1β-stimulated Human Umbilical Vein Endothelial Cells by Binding of Human Monocytic Cell Lines*

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Monocytes in the blood circulation migrate across endothelial cell monolayers lining the blood vessels and infiltrate into the underlying tissues in inflammation. However, little is known about the mechanisms by which leukocytes migrate across the endothelial barrier after binding and what molecules participate in the process. Addition of the human monocytic cell line THP-1 to interleukin-1β (IL-1β)-stimulated human umbilical vein endothelial cells (HUVEC) induced a decrease in the amount of focal adhesion kinase (p125FAK), a tyrosine kinase localized at focal contacts and essential for cell attachment to the extracellular matrix, whereas little change was observed in the amount of other molecules associated with cell adhesion such as vascular cell adhesion molecule-1 (VCAM-1), interleukin-1 receptor (IL-1R), and ICAM-1. A maximum decrease in the amount of p125FAK was observed 15–30 min after addition of THP-1 cells to HUVEC, after which the level of p125FAK gradually recovered. A reduction in the density of actin stress fibers in IL-1β-activated HUVEC was observed in parallel with the decrease in p125FAK. The p125FAK decrease was partially inhibited by preventing THP-1 binding to HUVEC using a mixture of antibodies to adhesion molecules. We suggest that the decrease in p125FAK triggered by binding of monocytes in inflammation facilitates the transendothelial migration of the monocytes by altering the adhesiveness of endothelial cells to the extracellular matrix.

In the early stages of inflammation, monocytes and other leukocytes in the blood circulation migrate across endothelial cell monolayers lining the blood vessels and enter the perivascular tissues. The migration of leukocytes involves multiple steps, and various types of adhesion molecules participate in these processes, including selectins mediating initial tethering and rolling of leukocytes over the endothelial cells, and integrins on leukocytes interacting with adhesion molecules belonging to the immunoglobulin superfamily expressed on the endothelial cells (1, 2). In acute inflammation, the expression of adhesion molecules is regulated by media tors such as thrombin, inflammatory cytokines, and chemokines (2).

Although many observations have focused on the molecules participating in the events from tethering to adhesion of leukocytes to endothelial cells, little is known about the mechanisms whereby leukocytes migrate across the endothelial barrier after binding and which molecules participate in the process.

Platelet/endothelial cell adhesion molecule-1 (PECAM-1) is one of the adhesion molecules that is concentrated at intercellular junctions between endothelial cells (3). Anti-PECAM-1 monoclonal antibody (mAb) or soluble PECAM-1 inhibits the transmigration of leukocytes through endothelial cell monolayers in vitro without interfering with the leukocyte’s potential to adhere tightly to the apical surface of endothelial cells (4). For neutrophils, integrin-associated protein (CD47) present on both neutrophils and endothelial cells is supposed to be essential for invasion (5). Activation of intercellular adhesion molecule-1 (ICAM-1) by binding of T cells has been reported to transduce a signal into endothelial cells, which induces tyrosine phosphorylation of the actin-binding protein cortactin, indicating alterations in the cytoskeleton (6). These findings suggest the possibility that binding itself induces changes in endothelial cells leading to relaxation of interendothelial cell junctions are significant.

To delineate the mechanism whereby monocytes can transmigrate through the endothelium during inflammation, we first investigated the changes in protein phosphorylation patterns of interleukin-1β (IL-1β)-stimulated human umbilical vein endothelial cells (HUVEC) overlayed with human mononuclear THP-1 cells and found that the addition of THP-1 cells induces a decrease in the amount of a phosphorylated 120–130-kDa protein(s) in HUVEC. In this study, we show that the decreased protein is focal adhesion kinase (p125FAK), a tyrosine kinase present at focal contact sites, and we discuss the possible involvement of this alteration in the process of leukocyte migration at sites of inflammation.

EXPERIMENTAL PROCEDURES

Cell Lines—HUVEC were purchased from Kurabo (Osaka, Japan) and were cultured on gelatin-coated culture flasks (Iwaki glass, Tokyo, Japan) with EGM-UV medium (Kurabo). Human monocytic THP-1 cells (Japanese Cancer Research Resources Bank, Tokyo, Japan), monoblas-

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† The abbreviations used are: PECAM-1, platelet/endothelial cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; HUVEC, human umbilical vein endothelial cells; p125FAK, focal adhesion kinase; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; anti-Tyr(P), anti-phosphotyrosine; mAb, monoclonal antibody; rt, room temperature; PBS, phosphate-buffered saline.
tic U937 cells (American Type Culture Collection, Rockville, MD), pro-
murineotypic HL-60 cells (Fujisaki Cell Center, Hayashibara Biochemical
Laboratories, Inc., Okayama, Japan), and T leukemic MOLT-16 cells (Fujisaki
Cell Center) were maintained in RPMI 1640 (Nissui Pharmaceutical,
Tokyo, Japan) supplemented with 10% fetal bovine serum (Life Tech-
ological Inc., Tokyo, Japan). 10 mM HEPES, 100 units/ml penicillin, and 50 µg/ml streptomycin.

Reagents—Human IL-1β was purchased from Genzyme (Cambridge,
MA). Human natural tumor necrosis factor-α (TNF-α) (specific activity of
2 × 10^10 Japan reference unit/ml) was prepared in our laboratory (7).
Mouse monoclonal anti-phosphotyrosine (anti-Tyr(P)) PY20, anti-
Tyrosine-120, anti-p125FAK, anti-C-20, and anti-integrin β3 were pur-
chased from Seikagaku Corp. (Tokyo, Japan). Rabbit polyclonal anti-
p125FAK, anti-p95FAK, anti-p130FAK, and anti-p120FAK were obtained from R&D Systems (Abingdon, UK), and anti-sialyl Lea CSLEX1 (IgM)
were purchased from Becton Dickinson (Bedford, MA). Anti-talin mAb
TA205 was purchased from Genosys (Cambridge, UK). Rabbit polyclon-
al anti-catenin was purchased from Sigma.

Preparation of Whole Cell Lysates, Cell Extracts, and Insoluble Fractions
of HUVEC—Six-cm culture dishes (Falcon 3002, Becton Dickin-
don) were coated with 4 ml of a 100 µg/ml solution of gelatin (Iwaki Glass) in
phosphate-buffered saline (PBS) for 2 h, and HUVEC were grown to confluency on the coated dishes. HUVEC were stimulated with
a washing buffer (1% Triton X-100, 1% Nonidet P-40, 150 mM NaCl, 2 mM
phenylmethylsulfonyl fluoride, 250 µM leupeptin, 2 mM EDTA, 50 mM
Tris, pH 7.5) with the aid of a cell scraper. The lysates were stood
on ice for 30 min with occasional mixing. One hundred µl of the lysates
were then transferred to new tubes as the whole cell lysate. The resid-
ual lysates were centrifuged at 13,000 × g for 30 min, and the super-
antats were collected and used as the cell extract. Four hundred µl of
an extraction buffer solutions containing 1% SDS were added to each
remaining pellet and were dissolved by vigorous pipetting. These frac-
tions were defined as the insoluble fraction.

Immunoprecipitation and Immunoblotting—The cell extracts were
incubated with 1 µg of anti-Tyr(P) 4G10 for 2 h or with 4 µg of
anti-p125FAK 2A7 for 16–18 h at 4 °C with continuous mixing. Protein
G-Sepharose (Pharmacia, Uppsala, Sweden) was washed twice with
Tris-buffered saline (150 mM NaCl, 10 mM Tris, pH 7.4) and once with
a washing buffer (1% Triton X-100, 1% Nonidet P-40, 150 mM NaCl, 50
mM Tris, pH 7.5), and the resin pellet was resuspended in washing
buffer. The resins from 100-µl volumes of 50% suspensions were mixed
with the HUVEC lysates and were incubated for 2 h at 4 °C with
continuous mixing. The resins were washed three times with washing
buffer and then resuspended in 40 µl of a 2 × SDS-sample buffer (100
mM Tris, 5% SDS, 30% glycerol, 5% 2-mercaptoethanol, pH 6.8), and
boiled for 10 min. After centrifugation, 20 µl of the supernatants were
subjected to electrophoresis on a 7.5% polyacrylamide gel in the pres-
ence of SDS and transferred to nitrocellulose filters. In the case of direct
immunoblotting, samples were treated with half their volume of 3
× SDS-sample buffer (150 mM Tris, 7.5% SDS, 45% glycerol, 7.5% 2-mer-
captoethanol, pH 6.8), and 20 µl of the treated samples were subjected
to electrophoresis.

After blocking nonspecific binding with Block Ace (Yukijirushi, Sappo-
oro, Japan), the filters were probed with the antibody of interest for
2–3 h at room temperature (rt), followed by either horseradish peroxi-
dase-labeled rabbit anti-rabbit Igs (Dako Japan, Kyoto, Japan), horse-
radish peroxidase-labeled swine anti-rabbit Igs (Dako Japan), or a
Vectorastain ABC-PO kit for goat IgG (Vector Laboratories, Burlingame,
CA) for 2–3 h at rt. Washing of the membranes was performed with
Tris-buffered saline containing 0.05% Tween 20. The bands were visu-
alized with the enhanced chemiluminescence detection system (Amer-
sham, Buckinghamshire, United Kingdom) as directed by the manufac-
turer. In the case of reprobing the same membranes with a different
first antibody, the horseradish peroxidase of the already bound second
antibody was removed by washing the blot with Block Ace supple-
mented with 0.1% NaNO₂ for 16–18 h at rt. Quantification of the density
of the detected bands was performed by scanning densitometry using
ImageMaster DTS (Pharmacia).

Fluorescence Microscopy—Poly styrene chamber slides (Nippon In-
terMed, Tokyo, Japan) were coated with gelatin for 2 h. HUVEC
were plated on the slides and cultured to confluency. HUVEC were then
stimulated with 0.5 ng/ml IL-1β for 5 h and were subsequently layered
with 1.5 × 10⁶ THP-1 cells at different periods. After removing
the supernatant and washing, the cells were fixed with a mixture of acetone
and methanol (1:1 v:v) for 20 min at −20 °C, and after washing with PBS,
the cells were incubated with 2.5 units/ml of rhodamine phalloidin
(Wako Pure Chemical Industries, Osaka, Japan) for 1 h at rt. After
washing, the slides were mounted using 50% glycerol in PBS and
observed under a fluorescence microscope (model BHF, Olympus, To-
kyo, Japan).

Cell Adhesion Assay—Ten thousand HUVEC were seeded in each
well of gelatin-coated 96-well culture plates (Iwaki Glass) and cultured
for 48 h. Confluent cultures of HUVEC were stimulated with IL-1β or
TNF-α. After washing the coated dishes with the treated samples from each well
or TNF-α for 5 h and then washed once with assay medium (RPMI
supplemented with 0.1% bovine serum albumin (Armiur Pharma-
cutical, Kankakee, IL), 10 mM HEPES, 100 units/ml penicillin, and 50 µg/ml streptomycin) before addition of isotope-labeled THP-1 cells.
THP-1 cells were labeled with 51CrO₄ (Amersham) at 37 °C for 1 h.
After washing three times with the culture medium, 5 × 10⁶ labeled
THP-1 cells suspended in the assay medium were added to each well in
100-µl volumes. In inhibition experiments using adhesion-blocking an-
tibodies, 51Cr-labeled THP-1 cells incubated with 50 µg/ml mAbs to
adhesion molecules for 60 min at rt and washed twice were used. After
mild centrifugation at 40 × g for 1 min, the plates were incubated at
37 °C for 30 min. The nonadherent cells were removed by washing twice
with the assay medium, and the adherent THP-1 cells were lysed with
1% Triton X-100. The radioactivity from samples of supernatants from each
well and the original THP-1 cell suspension was determined by gamma
counter, and the percentage of THP-1 cells adhering to HUVEC in each
well was calculated.

RESULTS

The p125FAK Level in IL-1β-stimulated HUVEC Is Decreased by Co-culture with Monocytic Cell Lines—First we investigated
the changes in the tyrosine phosphorylation levels of molecules in
HUVEC after adding human leukemic THP-1, U937, HL-60, or MOLT-16 cells. To simulate the activated state of blood
vessels in inflammation, HUVEC were pretreated with IL-1β for
5 h. After treating HUVEC with leukemic cells, the cells
were lysed with the extraction buffer, and cell extracts were
prepared. Tyrosine phosphorylation patterns were assessed by
immunoprecipitation with anti-Tyr(P) 4G10 and subsequent
immunoblotting with anti-Tyr(P) PY20. As shown in Fig. 1A,
several tyrosine-phosphorylated proteins were observed in IL-
1β-stimulated HUVEC in the absence of the leukemic cell lines
(lane 1). In the case of co-culture with THP-1 cells, a decrease
in tyrosine-phosphorylated 120–130-kDa proteins in IL-1β-
stimulated HUVEC was very obvious (lanes 2 and 3). Consid-
ering the molecular size and the levels of expression of the
phosphorylated molecule(s) observed in our experiments,
p125FAK was selected as a probable candidate for the tyrosine-
phosphorylated 120–130-kDa protein observed in HUVEC. To
confirm the identity of the protein(s) banding at 120–130 kDa,
cell extracts from HUVEC co-cultured with leukemic cell lines
were immunoprecipitated with anti-p125FAK 2A7 and probed
with anti-Tyr(P) PY20. As shown in Fig. 1B, anti-p125FAK 2A7
immunoprecipitated a 120–130 kDa protein, and the amount of
immunoprecipitated molecule(s) was reduced by THP-1 co-
culture in a manner depending on the number of seeded THP-1
cells (Fig. 1B, lanes 2 and 3). This indicates that a component of
the tyrosine-phosphorylated 120–130-kDa band is identical to
p125FAK.

The residual immunoprecipitates obtained by immunoprecipita-
tion with anti-p125FAK 2A7 were probed with anti-p125FAK
C-20. As shown in Fig. 1C, the pattern of immunobLOTS detected with polyclonal anti-p125FAK C-20 was similar identi-
cal to the pattern that was obtained with anti-Tyr(P) PY20,
indicating that the decrease in the amount of p125FAK protein itself and not from
tyrosine de-phosphorylation of the protein. To further clarify
the reason for the decrease in p125FAK, changes in the amount of
p125FAK in whole cell lysates, cell extracts, and in insoluble
fractions were investigated by direct immunoblotting with an-
lysates were immunoblotted directly with anti-p125 FAK. Gelatin-coated culture dishes were treated with 0.5 ng/ml IL-1β for 5 h and were subsequently overlaid with 6 × 10^5 cells/2 ml or 2 × 10^6 cells/2 ml of the human leukemic cells for 30 min. A, the cells were lysed with extraction buffer, and the cell extracts were subjected to immunoprecipitation with anti-Tyr(P) 4G10. The immunoprecipitates were then immunoblotted with anti-Tyr(P) PY-20. Molecular markers (kDa) are indicated on the left, and the tyrosine-phosphorylated 120–130-kDa bands (arrow) are indicated on the right. B, the cells were lysed with extraction buffer, and the cell extracts were subjected to immunoprecipitation with anti-p125 FAK 2A7. One-half of the immunoprecipitates were immunoblotted with anti-Tyr(P) PY-20. The position of the p125 FAK band is indicated on the right. C, the other half of the immunoprecipitates were immunoblotted with anti-p125 FAK C-20. The position of the p125 FAK band is indicated on the right. D, the cells were lysed with the extraction buffer, and the whole cell lysates were immunoblotted directly with anti-p125 FAK C-20. The position of the p125 FAK band is indicated on the right. IP, immunoprecipitation; IB, immunoblotting.

Decreased Protein Levels in HUVEC by Co-culture with THP-1 Cells—We investigated whether levels of proteins in IL-1β-activated HUVEC other than p125 FAK were reduced by THP-1 seeding or not. Talin present at focal contacts (8) such as p125 FAK expressed on the cell surface of cytokine-activated endothelial cells (9), and α-catenin co-localized at the sites of intercellular junctions with cadherin and β-catenin (10), were probed with their respective antibodies on the same transferred membrane. As shown in Fig. 2, no changes in the amount of VCAM-1 and α-catenin were observed in whole cell lysates of HUVEC co-cultured with THP-1 cells. In the case of talin, a slight decrease was observed, and a possible degradation fragment of approximately 200 kDa was identified. However, the extent of the decrease in talin was far less than that observed in p125 FAK. In U937-treated HUVEC, patterns for the probed proteins were almost the same as those observed in HUVEC treated with THP-1 cells (Fig. 2).

VCAM-1 and α-catenin were not detected in whole cell lysates obtained from 2 × 10^6 THP-1 cells alone, although talin was faintly detectable (lane 4). In subsequent experiments we included α-catenin as a control to show that equal amounts of HUVEC protein were included in each sample of our assays.

Kinetics of the Decrease in the Amount of p125 FAK in IL-1β-treated HUVEC—To further characterize the decrease in the amount of p125 FAK, the kinetics of the changes in p125 FAK after...
addition of THP-1 cells were investigated. As shown in Fig. 3, the decrease in p125FAK was detected from 5 min after the addition of THP-1 cells (lane 5) and reached a maximum 15–30 min later (lanes 6 and 7) in IL-1β-stimulated HUVEC. Although the amount of p125FAK did not return to initial levels, a tendency for recovery of p125FAK was observed (lane 10); 4 h from THP-1 cell seeding. Interestingly, the p125FAK degradation was not observed in unstimulated HUVEC (lanes 1–3).

Changes in the Cytoskeletal Structure of HUVEC Induced by THP-1 Binding—To investigate whether THP-1 treatment induces changes in the cytoskeletal structure of HUVEC, the HUVEC were stimulated with or without IL-1β for 5 h and were subsequently overlaid with THP-1 cells. The cells were fixed, stained with rhodamine phalloidin, and observed by fluorescence microscopy. Well organized actin stress fibers were observed in both unstimulated (Fig. 4A) and IL-1β-stimulated HUVEC (Fig. 4E) before seeding of THP-1 cells. The well-developed stress fibers were also observed in unstimulated HUVEC which were overlaid with THP-1 cells (Fig. 4B–D). In contrast, THP-1 seeding markedly reduced the number, thickness, and length of actin stress fibers in HUVEC preactivated with IL-1β (Fig. 4, F–H). The changes were observed from 30 min after seeding THP-1 cells and continued for at least 2 h.

Effect of IL-1β and TNF-α Treatment of HUVEC on the Decrease in p125FAK in IL-1β-stimulated HUVEC—The decrease in the amount of p125FAK induced by THP-1 cells was observed in IL-1β-treated HUVEC but not in unstimulated HUVEC. Therefore, we investigated further whether THP-1 cells could induce the decrease in p125FAK in HUVEC stimulated with inflammatory stimuli other than IL-1β. HUVEC were stimulated with TNF-α for 5 h and were then co-cultured with THP-1 for 30 min. The cells were lysed and subjected to immunoblotting with anti-p125FAK C-20. As shown in Fig. 5A, decrease in the amount of p125FAK after addition of THP-1 was observed not only in IL-1β-stimulated HUVEC (lanes 2–5) but also in TNF-α-activated HUVEC (lanes 7–10) in a manner dependent on the concentration of the cytokines added. We simultaneously investigated the binding of THP-1 cells to HUVEC activated with these inflammatory stimuli. 51Cr-labeled THP-1 cells were added to activated HUVEC and incubated for 30 min. As shown in Fig. 5B, it was observed that IL-1β and TNF-α treatment of HUVEC augmented THP-1 binding to HUVEC in a dose-dependent manner, suggesting a correlation between decrease in p125FAK and binding of THP-1 cells to cytokine-activated HUVEC.

Inhibition of the Decrease in p125FAK by Adhesion-blocking mAbs—It is well known that IL-1β and TNF-α induce the expression of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin on the surface of endothelial cells (9, 11, 12). Therefore, we investigated whether pretreating THP-1 cells with a blocking antibody to the counter-receptors for ICAM-1, VCAM-1, or E-selectin could inhibit the decrease in p125FAK or not. THP-1 cells have been reported to express β2 integrin, a β subunit of the β2 integrin family, α4 integrin, an α subunit of very late antigen-4, and sialyl Leα (13). β2 integrins, very late antigen-4, and sialyl Leα are known to interact with ICAM-1, VCAM-1, and E-selectin, respectively (14–18). THP-1 cells were incubated with 50 μg/ml anti-α4, anti-β2, anti-sialyl Leα, or a mixture of these three mAbs at rt for 1 h. After washing three times with medium, the antibody-pretreated THP-1 cells were seeded over IL-1β-activated HUVEC. As shown in Fig. 6A, pretreatment of THP-1 cells with a mixture of anti-α4, anti-β2, and anti-sialyl Leα inhibited the decrease in p125FAK in IL-1β-activated HUVEC (lane 6), whereas treatment with either of these mAbs alone could not inhibit the decrease in p125FAK (lanes 3–5). Fig. 6B shows the result of quantification of the density of the detected blots in Fig. 6A. In the case of cell adhesion assays, only treatment with a mixture of the three
with IL-1β, HUVEC grown on a gelatin-coated 96-well microplate were treated with IL-1β (lanes 2–5) or TNF-α (lanes 7–10) for 5 h. Subsequently, 1 × 10⁵ THP-1 cells were added and incubated for 30 min. The cells were lysed with extraction buffer, and the whole cell lysates were subjected to immunoblotting with anti-p125FAK C-20. Subsequently, α-catenin on the same membrane was also probed. The position of the p125FAK and α-catenin bands are indicated on the right. B, HUVEC grown on a gelatin-coated 96-well microplate were treated with IL-1β or TNF-α for 5 h. ⁵¹Cr-labeled THP-1 cells were added to the activated HUVEC and incubated for 30 min. Nonadherent cells were removed, and the adherence of THP-1 cells was determined. Values shown represent the mean ± S.D. of triplicate wells.

mAbs similarly inhibited the adherence of THP-1 cells to activated HUVEC (Fig. 6C, lane 6).

**DISCUSSION**

Focal contacts are regions of the cell that come in direct contact with the extracellular matrix, providing anchorage sites for actin stress fibers and forming a link between the extracellular matrix and the actin cytoskeleton (19). The p125FAK molecule is a tyrosine kinase co-localized in focal contacts with several other molecules, such as talin and tensin (20–22), and plays a central role in integrin-mediated signal transduction from the extracellular matrix (20–24). In this study, we showed that binding of THP-1 cells to IL-1β-stimulated HUVEC induces a decrease in the amount of the p125FAK molecule in HUVEC. It has been reported that inhibition of the function of p125FAK by p41/43FRNK (pp125FAK-related non-kinase) blocked the formation of focal contacts, indicating a functional relation between p125FAK and the formation of focal contacts (25). Moreover, the loss of p125FAK has been reported to be a prerequisite for cell detachment (26). Taken together, it was considered that the decrease in p125FAK in HUVEC indicates a decrease in the function of focal contacts, resulting in decreased strength of attachment of the endothelial cell to the extracellular matrix. The decrease in the adhesiveness of endothelial cells would enable monocytes to migrate beneath the endothelial cells more easily.

A decrease in the density of actin fibers induced by THP-1 was also observed in HUVEC in parallel with the decrease in p125FAK. It has been well documented that the formation of actin stress fibers parallels the formation of focal adhesion and is accompanied by increased tyrosine phosphorylation of p125FAK (27–29). Integrity of the actin cytoskeleton has also been reported to be required for the increased phosphorylation of p125FAK in response to a variety of extracellular stimuli (23, 30). Therefore, it can be postulated that the decrease in actin stress fibers is closely associated with the decrease in p125FAK.

It is unclear why the p125FAK protein level drops so rapidly. Recently, it was reported that p125FAK is cleaved by calpain, a calcium-dependent cysteine protease, in platelets (31). Therefore, we investigated whether the decrease in p125FAK could be prevented by calpeptin, a membrane-permeable inhibitor of calpain, or a cysteine protease inhibitor E-64. However, pretreatment of HUVEC by these inhibitors at a concentration of up to 50 μM could not affect the decrease in p125FAK (data not shown). In addition, little change was observed in the amount of talin which interacts with p125FAK (32) and is cleaved by calpain preferentially (33). From these results, it is unlikely that calpain is responsible for the decrease in p125FAK. The molecular mechanisms of the decrease in p125FAK are still inconclusive, even though we have also tried to inhibit the decrease in p125FAK by other protease inhibitors.

The decrease in p125FAK in IL-1β-stimulated HUVEC was...
induced not only by monocyctic THP-1 cells but also by monoblastic U937 cells. Furthermore, the decrease induced by THP-1 was also observed in HUVEC grown on collagen type I or fibronectin (data not shown), indicating that the decrease in p125<sub>FAK</sub> was independent of the extracellular matrix on which the HUVEC were grown. These results indicate that the decrease in the amount of tyrosine-phosphorylated p125<sub>FAK</sub> might be a commonly observed event in cytokine-activated HUVEC.

The molecules participating in the interactions between THP-1 cells and HUVEC remain to be clarified. The candidate molecule that triggers the decrease in p125<sub>FAK</sub> is considered to be an adhesion molecule present on THP-1 cells rather than a newly secreted soluble factor induced by interaction of THP-1 cells with activated HUVEC because the cell-free culture supernatant obtained after co-culture of THP-1 cells with IL-1β-activated HUVEC did not induce a decrease in p125<sub>FAK</sub> levels (data not shown). With regard to the counter-receptor(s) on the surface of HUVEC responsible for the transduction of the p125<sub>FAK</sub>-modifying signal, although the decrease in the amount of p125<sub>FAK</sub> was partially blocked by a mixture of neutralizing mAbs against ICAM-1, VCAM-1, and E-selectin pathways, a direct role for these three adhesion molecules in the transmission of a regulatory signal has yet to be established. It is possible that adhesion molecules such as ICAM-1, VCAM-1, and E-selectin, the expression of which is augmented by inflammatory cytokines, enable THP-1 cells to bind tightly to HUVEC, resulting in the effective transduction of the p125<sub>FAK</sub> reducing signal induced by other molecule(s) into HUVEC.

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