Journal of Cellular Biochemistry 64:505–513 (1997)

Cyclic Strain Induces Reorganization of Integrin $\alpha_5\beta_1$ and $\alpha_2\beta_1$ in Human Umbilical Vein Endothelial Cells

Yoshiko Yano, John Geibel, and Bauer E. Sumpio*

Department of Surgery, Yale University School of Medicine, New Haven, Connecticutt

Abstract Cyclic strain has been shown to modulate endothelial cell (EC) morphology, proliferation, and function. We have recently reported that the focal adhesion proteins focal adhesion kinase (pp125^{FAK}) and paxillin, are tyrosine phosphorylated in EC exposed to strain and these events regulate the morphological change and migration induced by cyclic strain. Integrins are also localized on focal adhesion sites and have been reported to induce tyrosine phosphorylation of pp125^{FAK} under a variety of stimuli. To study the involvement of different integrins in signaling induced by cyclic strain, we first observed the redistribution of α and β integrins in EC subjected to 4 h cyclic strain. Human umbilical vein endothelial cells (HUVEC) seeded on either fibronectin or collagen surfaces were subjected to 10% average strain at a frequency 60 cycles/min. Confocal microscopy revealed that β_1 integrin reorganized in a linear pattern parallel with the long axis of the elongated cells creating a fusion of focal adhesion plaques in EC plated on either fibronectin (a ligand for $\alpha_5\beta_1$) or collagen (a ligand for $\alpha_2\beta_1$) coated plates after 4 h exposure to cyclic strain. β_3 integrin, which is a vitronectin receptor, did not redistribute in EC exposed to cyclic strain. Cyclic strain also led to a reorganization of α_5 and α_2 integrins in a linear pattern in HUVEC seeded on fibronectin or collagen, respectively. The expression of integrins α_5, α_2, and β_1 did not change even after 24 h exposure to strain when assessed by immunoprecipitation of these integrins. Cyclic strain-induced tyrosine phosphorylation of pp125^{FAK} occurred concomitant with the reorganization of β_1 integrin. We concluded that $\alpha_5\beta_1$ and $\alpha_2\beta_1$ integrins play an important role in transducing mechanical stimuli into intracellular signals. J. Cell. Biochem. 64:505–513.

Key words: cyclic strain; human umbilical vein endothelial cell; integrin; focal adhesion kinase; fibronectin; collagen type I

Endothelial cells (EC) form the inner lining of blood vessels and are exposed to a continually changing hemodynamic environment. We have previously utilized an apparatus that can apply cyclic strain to an attached monolayer of EC to approximate the repetitive distension of the endothelium in vivo [Gilbert et al., 1989; Banes et al., 1990; Sumpio et al., 1987]. The use of this in vitro model has demonstrated that cyclic strain alters EC proliferation [Li et al., 1994; Sumpio et al., 1987] and changes the morphology of EC from a polygonal shape to elongated shape, with their long axes aligned perpendicular to the strain force [Iba et al., 1991; Yano et al., 1996a].

In vivo, EC normally adhere to a basement membrane composed of different matrix proteins, such as fibronectin, laminin, vitronectin, and collagen [Herman et al., 1987]. Integrins are extracellular matrix (ECM) receptors, which are concentrated in focal adhesions [Chen et al., 1985] and are capable of binding to various cytoskeletal proteins [Horwitz et al., 1986]. The $\alpha_5\beta_1$ (fibronectin receptor), $\alpha_v\beta_3$ (vitronectin receptor), and $\alpha_2\beta_1$ (collagen receptor) integrins have been identified in EC [Kirchhofer et al., 1990; Fath et al., 1989; Dejana et al., 1988; Albelda et al., 1989] and recent studies have shown that integrin clustering, either with ligand or with antibodies induces the tyrosine phosphorylation of focal adhesion kinase (pp125^{FAK})[Lipfert et al., 1992; Kornberg et al., 1992].

Recently we reported that exposure of bovine EC to cyclic strain (60 cycle/min, 10% average strain) induced significant tyrosine phosphory-

Contract grant sponsor: NIH, contract grant number R01 HL 47345; Contract grant sponsor: Veterans Administration Review Board; Contract grant sponsor: American Heart Association (National).

*Correspondence to: Bauer E. Sumpio, M.D., Ph.D., Department of Surgery, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510. E-mail: bauer.sumpio@yale.edu

Received 25 June 1996; Accepted 9 October 1996

lation of focal adhesion proteins especially pp125^{FAK} and paxillin and these proteins regulate the morphological changes and increased migration induced by cyclic strain [Yano et al., 1996a]. We also reported that the strain-induced tyrosine phosphorylation of these proteins is regulated by small GTP-binding protein rho [Yano et al., 1996b]. Since we observed that cyclic strain led to reorganization of the focal adhesions [Yano et al., 1996a], we hypothesized that integrins play critical roles in the strain response of EC. In the present study we show that cyclic strain results in reorganization, but not expression level, of specific integrins in focal adhesions of EC depending on the substrate. We speculate that the α-subunit acts as a mechanotransducer by sensing and responding to cyclic strain with subsequent transduction of the signal inside the cell through the cytoplasmic domain of β_1 integrin.

MATERIAL AND METHODS

Culture of Human Umbilical Vein Endothelial Cells

Human umbilical vein endothelial cells (HUVEC) were obtained from the American Type Tissue Collection (Rockville, MD). HUVEC were also harvested from fresh human umbilical vein using previously described techniques [Gimbrone et al., 1974]. Both cell types responded in an identical manner. Cells were cultured on plates that had been preincubated with 1% gelatin and maintained in Medium 199 (BioWhittaker, Inc., Walkersville, MD) supplemented with 20% heat-inactivated fetal bovine serum (Hyclone Laboratories, Inc., Logan, UT), 100 μg/ml heparin (Sigma Chemical Co., St. Louis, MO), 30 μg/ml endothelial cell growth supplement from bovine pituitary (Sigma Chemical Co.), 100 μg/ml L-glutamine, 1% penicillin, streptomycin, and amphotericin B (GIBCO BRL, Gaithersburg, MD) and grown to confluence at 37°C in a humidified 5% CO_2 incubator. HUVEC were identified by their typical cobble stone appearance and positive uptake of di-I-acetylated-LDL (Biomedical Technologies Inc., Stoughton, MA).

In Vitro Application of Cyclic Strain

HUVEC were seeded on flexible-bottomed 25 mm culture plates coated with fibronectin or collagen I (Flex I plate, Flexcell Corp., McKeesport, PA). Mechanical deformation was induced with a Flexercell Strain Unit (Flexcell Corp.) which consists of vacuum manifold regulated by solenoid valves that are controlled by a computer timer program. When vacuum is applied to the culture plate, the bottoms are deformed to a known percentage elongation [Banes et al., 1985, 1990; Gilbert et al., 1990, 1994]. For these experiments, the membrane bottoms were subjected to 150 mmHg vacuum, which produces an average strain of 10% on attached cells, at a rate of 60 cycles/min (0.5 s deformation alternating with 0.5 s in the neutral position). With this vacuum deformation, cells in the periphery (more than 5 mm from the center) experience 7–24% strain, whereas the cells in the center (within 5 mm of the middle of the membrane) experience less than 7% strain [Gilbert et al., 1989]. Unstretched cells grown on Flex I plates were used as controls.

Cell Surface Protein Biotinylation and Immunoprecipitation

At the end of the strain regimen, cells were washed with phosphate-buffered saline (PBS) and detached with PBS containing 2 mM EDTA at 37°C for 5 min. The cells were pelleted at 1,000*g* for 5 min and were washed with PBS. Sulfo-NHS-Biotin (Pierce Chemical Co., Rockford, IL) was added to a final concentration of 100 μg/ml and the cell suspension was incubated for 1 h. The cells were subsequently washed with PBS and lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1% NP-40, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) was added. Lysates were centrifuged at 15,000*g* for 10 min. For immunoprecipitation of the integrins, aliquots of supernatants containing equal amount of protein were incubated with anti-β_1 integrin, anti-α_2 integrin (Upstate Biotechnology Inc., Lake Placid, NY) or anti-α_5 integrin (GIBCO BRL, Gaithersburg, MD) followed by additional incubation with rabbit anti-mouse IgG and protein A-Sepharose (Pharmacia LKB Biotechnology Inc., Uppsala, Sweden). The samples were subjected to electrophoresis on SDS/7.5% polyacrylamide gels and subsequently transferred to nitrocellulose membranes. Blots were incubated with horse radish peroxidase-conjugated streptavidine (Amersham Corp., Arlington Heights, IL) and immunoreactivity was determined by enhanced chemiluminescence (ECL, Amersham Corp.). Immunoprecipitation of pp125^{FAK} was carried

out as described previously utilizing anti-pp125^{FAK} antibody (Transduction Laboratories Inc., Lexington, KY)[Yano et al., 1996a]. Blots were labeled by anti-phosphotyrosine antibody (py-20, Transduction Laboratories Inc.) to detect tyrosine phosphorylation level of pp125^{FAK}. All the bands were quantitated with Visage 2000 densitometer (BioImage, Ann Arbor, MI).

Confocal Microscopy

After exposure to the strain regimen, EC were fixed in 3% paraformaldehyde for 30 min, preincubated with 1% BSA in PBS for 1 h at room temperature, then incubated with anti-β_1 integrin, anti-α_2 integrin, or anti-α_5 integrin for 1 h at room temperature. Cells were washed three times in PBS and then incubated with fluorescein-labeled affinity purified goat anti-mouse IgG (Organon Teknika Corp., Durham, NC) for 1 h at room temperature. Samples were viewed with a confocal microscope (Axiovert 100, Carl Zeiss Inc., Thornwood, NY) with the focus on the cell attachment to the underlying membrane.

Statistics

Data are presented as the mean ± SD. Statistical analysis was performed by a paired *t*-test with a *P* value of <0.05 considered significant.

RESULTS

Redistribution of Integrins Induced by Cyclic Strain

Under static conditions, α_5 integrin, which is a component of the fibronectin receptor, localized in a random speckle pattern resembling focal contacts in HUVEC grown on either fibronectin (Fig. 1A) or collagen I (Fig. 1B). After 4 h exposure to strain, HUVEC grown on fibronectin were elongated and α_5 integrin was more concentrated and aligned along the long axis of the cells creating a large fusion of focal contacts (Fig. 1C). In contrast, in cells grown on the collagen coated membrane, α_5 integrin maintained a random speckle pattern, although the cells were elongated (Fig. 1D).

In HUVEC grown on fibronectin coated plates, α_2 integrin, a component of the collagen receptor, maintained a diffuse pattern with an occasional speckle concentration in some regions, in both the static or 4 h stretch group (Fig. 1E, G). Under static condition in HUVEC grown on collagen coated plates, α_2 integrin also maintained homogeneous speckle distribution that resembled focal contacts (Fig. 1F). However, after exposure of HUVEC grown on the collagen surface to cyclic strain, α_2 integrin seems to be more concentrated and reorganized in a linear fashion along the long axis of the elongated cells (Fig. 1H).

Since the fibronectin receptor ($\alpha_5\beta_1$) and collagen receptor ($\alpha_2\beta_1$) share the β_1 subunit, the distribution of β_1 integrin was investigated. In static HUVEC grown on either fibronectin or collagen I coated membranes, β_1 concentrates on regions resembling focal contacts (Fig. 2A, B). After 4 h exposure to strain, β_1 integrin seems to be brighter and its distribution aligned to the elongated long axis of cells (Fig. 2C, D).

As β_3 integrin, which is a component of vitronectin receptor ($\alpha_v\beta_3$), has also been identified in HUVEC [Newman et al., 1986; Plow et al., 1986], the distribution of β_3 integrin was investigated. In static cells grown on either fibronectin or collagen coated surface, β_3 integrin demonstrated a punctate distribution (Fig. 2E, F). After cells were exposed to 4 h strain, cells on either surface were elongated but there was no change in the distribution of β_3 integrin seen (Fig. 2G, H).

Effect of Strain on the Expression of Cell Surface $\alpha_5\beta_1$ AND $\alpha_2\beta_1$

Since the stretched cells show brighter staining than static cells in Figures 1C, H and 2C, D, immunoprecipitation of α_5, α_2, and β_1 integrin were performed to determine whether the level of expression of cell surface $\alpha_5\beta_1$ and $\alpha_2\beta_1$ was altered in response to cyclic strain. As shown in Figure 3A, α_5 integrin expression level does not change significantly after 24 h of stretch ($P > 0.5$, n = 5) although HUVEC grown on fibronectin coated plates have a higher expression level in either group compared to HUVEC grown on collagen I coated plates (2.0 ± 0.2-fold, n = 5). These data are consistent with the previous reports that ligand binding of $\alpha_5\beta_1$ integrin alters the integrin life cycle and permits a longer retention of these proteins at the cell surface [Dalton et al., 1995].

Similarly, the expression of α_2 (Fig. 3B) or β_1 integrin (Fig. 3C) did not change significantly in static or stretched cells grown on either fibronectin or collagen I ($P > 0.5$, n = 5).

Thus, cyclic strain induces the reorganization of specific integrins depending on the ligand in the absence of any significant changes in expression levels.

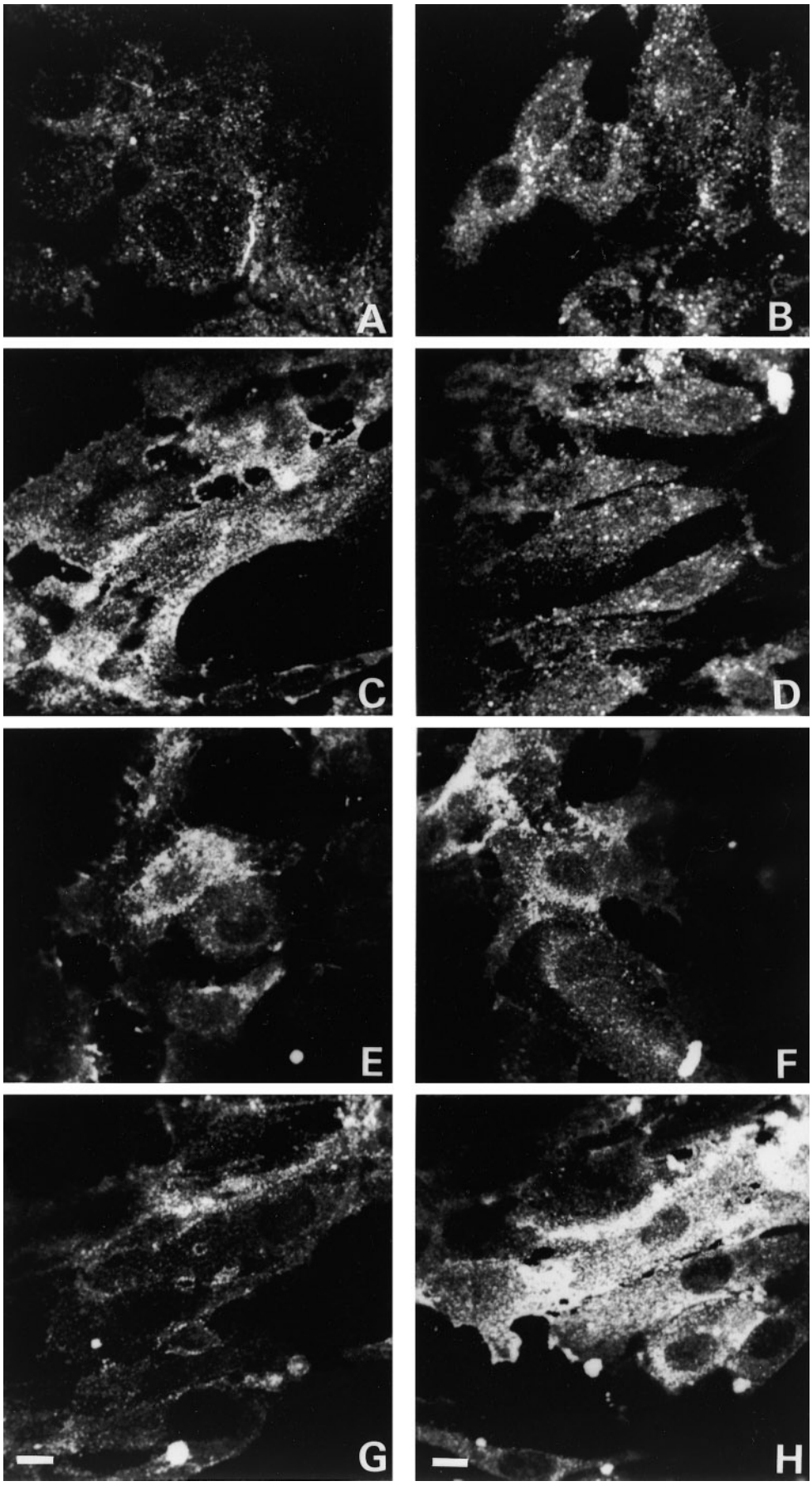

Figure 1.

Effect of Cyclic Strain on Tyrosine Phosphorylation of pp125^{FAK}

4 h exposure to cyclic strain increased tyrosine phosphorylation of pp125^{FAK} in HUVEC grown on the either fibronectin or collagen I (3.0 ± 1.0-fold, 3.9 ± 2.5-fold ($n = 5$))(Fig. 4A). These results confirm our previous findings utilizing bovine aortic EC [Yano et al., 1996a] although the magnitude of the response is less.

DISCUSSION

In the "tensegrity model" proposed by Ingber, any external mechanical force acting on the cells is balanced between tensile actin filaments and the ECM anchoring proteins in the focal adhesion plaque [Ingber et al., 1985; Ingber, 1991]. Since integrins play a pivotal role in maintaining this equilibrium [Ingber et al., 1985; Ingber, 1991], they represent potential mechanochemical transducers. We report here for the first time that cyclic strain reorganizes and fuses integrins in HUVEC (Fig. 1, 2) without affecting their levels of surface expression (Fig. 3A–C). Although cyclic strain did not change the expression level of α_5 integrin, HUVEC on fibronectin express more α_5 integrins than those on collagen I (Fig 3A). Other groups have observed the same phenomenon and speculate that since integrin metabolism involves the continuous synthesis and transport to the cell surface with subsequent internalization/degradation, integrin binding to extracellular matrix protein interrupts the cycle by blocking the internalization/degradation stage and permitting the retention of integrins at the cell surface [Dalton et al., 1995].

Although we did not detect any change in expression of β_1, Girard et al. reported that a different mechanical force, shear stress, induced a slight increase (two-fold) in integrin $\alpha_5\beta_1$ expression in bovine aortic EC exposed to shear stress for 24 h but not 6 h [Girard et al., 1995]. However, since the morphological change and alignment of F-actin in EC start to appear after 3 h exposure to shear stress [Girard et al., 1995], it is more likely that spatial redistribution, and not the expressions, of β_1 integrin play a more important role in early signaling events such as tyrosine phosphorylation and cytoskeletal reorganization. Our data is consistent with this thesis since integrin reorganization starts at the same time as when the morphological change and increase in tyrosine phosphorylation of pp125^{FAK} and paxillin are observed (Fig. 4)[Yano et al., 1996a]. As $\alpha_5\beta_1$ integrin has no intrinsic enzymatic activity and is itself not phosphorylated [Damsky et al., 1992], it is most likely that the activation and recruitment of associated cytoskeletal elements and tyrosine kinases such as pp125^{FAK} initiate the biochemical signaling [Schaller et al., 1994]. Moreover, we could not find significant increase in tyrosine phosphorylation of β_1 integrin induced by cyclic strain (data not shown).

Several investigators have reported that individual matrix ligands only induce the clustering of their respective membrane receptors and the integrins do not localize in focal contacts unless they are bound to their extracellular matrix [Sonnenberg, 1993; Dejana et al., 1988]. The $\alpha_5\beta_1$ fibronectin receptor (but not $\alpha_2\beta_1$ collagen I receptor) would be expected to localize in focal contacts when HUVEC are grown on fibronectin, whereas only the $\alpha_2\beta_1$ receptor localizes in focal contacts when cells are growing on collagen I substrates. When HUVEC are plated on fibronectin, the β_1 integrins are clustered but the β_3 integrins are not clustered [Dejana et al., 1988]. In our experiments, the α_5 integrin displays a speckle pattern resembling the focal adhesions in HUVEC on both fibronectin and collagen I (Fig. 1A, B). This discrepancy can be partially accounted for by the difference of antibodies that were used, but mainly by the difference in culture condition. Dejana et al. conducted their experiments in the absence of serum, while we conducted the present study in the presence of 20% serum which contains fibronectin and vitronectin [Hayman et al., 1983; Hayman et al., 1985; Thom et al., 1979]. Nonetheless after exposure of HUVEC to cyclic strain, fusion and redistribution of a specific integrin

Fig. 1. Spatial redistribution of α_5 and α_2 integrin induced by cyclic strain. HUVEC were seeded on fibronectin (**A,C,E,G**) or collagen I (**B,D,F,H**) coated membrane and were subjected to cyclic strain for 4 h (C,D,G,H) or maintained in stationary culture conditions (A,B,E,F). HUVEC were stained with anti-α_5 (A-D) or anti-α_2 integrin (E–H) and observed by confocal microscopy. In the static group, the cells cultured on fibronectin (A,E) or collagen (B,F) show a random speckle distribution of α_5 and α_2 integrin. After 4 h exposure to cyclic strain, cells were elongated and aligned perpendicular to the force vector (from left upper to right bottom). α_5 integrin in the cells grown on fibronectin (C) and α_2 integrin in the cells grown on collagen I (H) reorganized in a linear fashion, although α_5 integrin in the cells on collagen (D) and α_2 integrin in the cells on fibronectin (G) show a random punctate distribution similar to those in the static cells (A,B,E,F). Scale bar = 10 μm.

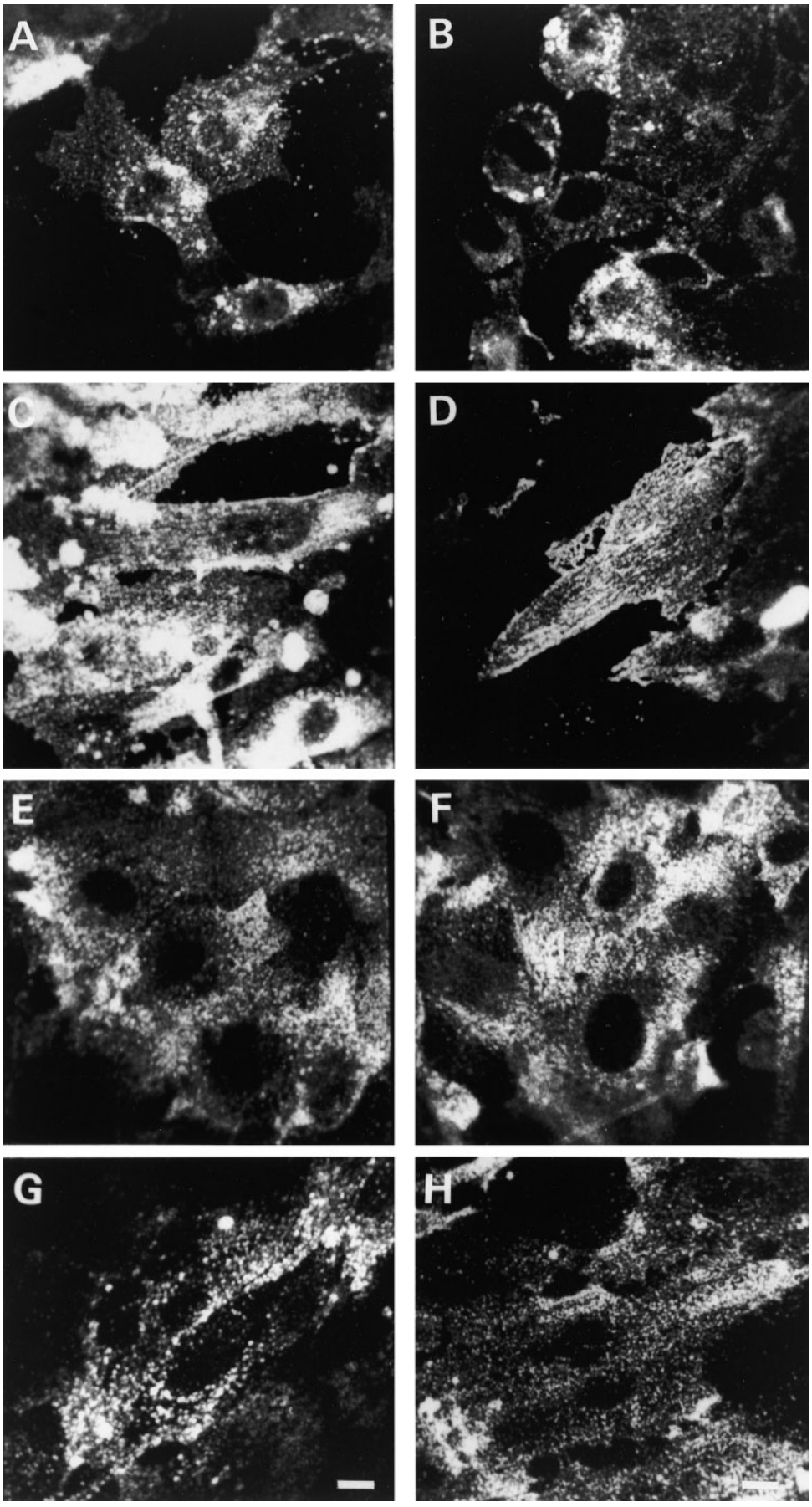

Figure 2.

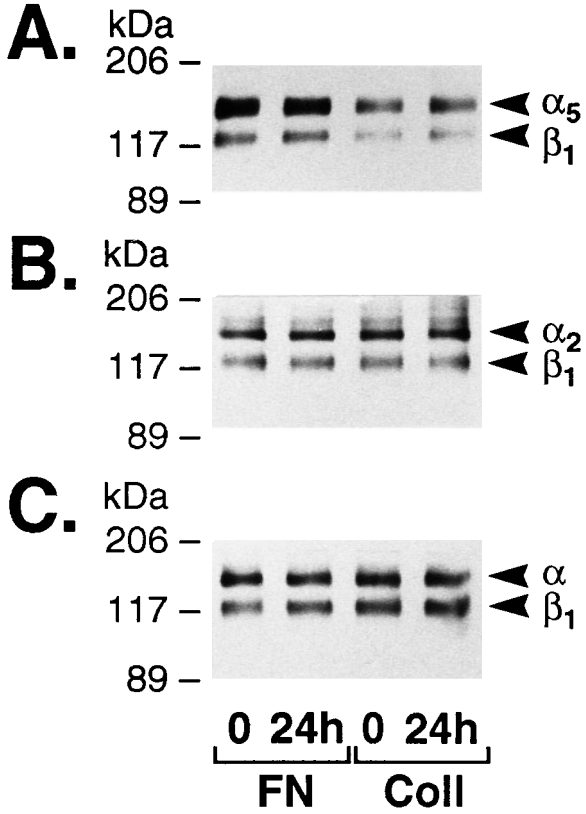

Fig. 3. Immunoprecipitation of integrin complexes with monoclonal antibodies. HUVEC were seeded on fibronectin (FN; **lanes 1 and 2**) or collagen I (Coll; **lanes 3 and 4**) and were exposed to cyclic strain for 24 h (lanes 2 and 4) or left in stationary culture (lanes 1 and 3). Cell extracts of surface biotin-labeled HUVEC were processed for immunoprecipitation using anti-α_5 (**A**), anti-α_2 (**B**), and anti-β_1 (**C**), and subjected to SDS-PAGE under nonreducing condition as described in Material and Methods. Cyclic strain did not alter the expression of α_5 (A), α_2 (B), and β_1 (C) significantly, although cells grown on fibronectin expressed significantly greater amounts of α_5 integrin than cells on collagen (A).

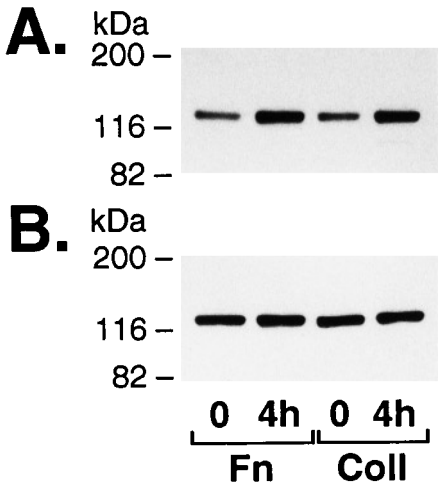

Fig. 4. Tyrosine phosphorylation of pp125^{FAK} induced by cyclic strain. HUVEC seeded on either fibronectin (Fn; **lanes 1 and 2**) or collagen I (Coll; **lanes 3 and 4**) substrates were subjected to strain for 4 h (lanes 2 and 4) or left in stationary culture (lanes 1 and 3). pp125^{FAK} was immunoprecipitated from cell lysates with anti-pp125^{FAK} antibody and immunoprecipitates were analyzed by immunoblotting with py-20 (**A**) or anti-pp125^{FAK} (**B**). Cyclic strain increased tyrosine phosphorylation of pp125^{FAK} in HUVEC grown on either fibronectin or collagen I.

could be observed only when HUVEC were seeded on the specific ligand which match the receptors respectively (Figs. 1 and 2).

Recent studies have shown that clustering of β_1 integrin induces the tyrosine phosphorylation of pp125^{FAK} [Kornberg et al., 1991; Kornberg et al., 1992; Lipfert et al., 1992]. Consistent with this observation, exposure of HUVEC to cyclic strain also induced tyrosine phosphorylation of pp125^{FAK} concomitant with the reorganization of β_1 integrin either on fibronectin or collagen I (Fig. 4). Considering the recent reports which indicate that the β_1 integrin chimera is capable of localizing in focal adhesions and inducing tyrosine phosphorylation of pp125^{FAK} [Geiger et al., 1992; LaFlamme et al., 1992], our observation on the clustering and reorganization of β_1 integrin by strain suggest that β_1 subunit is involved in the increase in strain-induced tyrosine phosphorylation of pp125^{FAK} followed by the change of morphology and migration. Although we tested whether antibodies directed against α_5, α_2, or β_1 could inhibit tyrosine phosphorylation or morphological change, no differences could be observed between those in the presence or absence of antibodies (data not shown). This finding may be partially accounted for by the fact that the antibodies were added after cells were already attached to the substrate and most of the integrin receptors were already occupied by specific ligands at that time. Therefore, under these condition, blocking effects of antibody were not sufficient enough to block these events.

Our study could not directly dissect the individual role of the α and β subunits in response

Fig. 2. Spatial redistribution of β_1 and β_3 integrin induced by cyclic strain. HUVEC were stained with anti-β_1 (**A–D**) and anti-β_3 (**E–H**) antibodies. Static cells grown on fibronectin (A,E) or collagen I (B,F) show a random speckle distribution of β_1 and β_3 integrin. After 4 h exposure of HUVEC to cyclic strain, β_1 integrin reorganized in a linear fashion along the long axis of the elongated cells in cells grown on either fibronectin (C) or collagen (D). β_3 integrin distribution remained unchanged (G,H). Cell alignment is perpendicular to the strain vector (from upper left to bottom right). Scale bar = 10 μm.

to the cyclic strain. However, the associated α subunit, which determines the binding specificity of the receptor complex to substrate [Buck et al., 1986; Kishimoto et al., 1987; Ruoslahti et al., 1987] is likely to play an important role as sensor and regulator of the mechanical stimuli on the specific ligand since the engagement of the α-subunit with its specific substrate determines which integrin complex localizes in the focal adhesions [Ruoslahti et al., 1987; Kishimoto et al., 1987; Buck et al., 1986]. This in turn regulates the interaction between the cytoskeletal proteins and the β_1 cytoplasmic domain [LaFlamme et al., 1992].

In conclusion, our data support the role of the integrin as a mechanotransducer, which is capable of coupling the outside forces on a cell to biochemical signals in the focal adhesion that potentially transmit these signals to the rest of the cell.

ACKNOWLEDGMENTS

This work was supported in part by grants to BES from the NIH (R01 HL 47345), the Veterans Administration Review Board and the American Heart Association (National).

REFERENCES

Albelda SM, Daise M, Levine EM, Buck CA (1989): Identification and characterization of cell-substratum adhesion receptors on cultured human endothelial cells. J Clin Invest 83:1992–2002.

Banes AJ, Gilbert J, Taylor D, Monbureau OA (1985): A new vacuum-operated stress providing instrument that applies static or variable duration cyclic tension or compression to cells in vitro. J Cell Sci 75:35–37.

Banes AJ, Link GW, Gilbert JW, Son Tay RT, Monbureau O (1990): Culturing cells in a mechanically active environment. Am Biotech Lab 8:12–22.

Buck CA, Shea E, Kimberly D, Horwitz AF (1986): Integrin (the CSAT antigen): Functionality requires oligomeric integrity. J Cell Biol 103:2421–2428.

Chen WT, Hasegawa T, Hasegawa C, Weinstock C, Yamada KM (1985): Development of cell surface linkage complexes in cultivated fibroblasts. J Cell Biol 100:1103–1114.

Dalton SL, Scharf E, Briesewitz R, Marcantonio EE, Assoian RK (1995): Cell adhesion to extracellular matrix regulates the life cycle of integrins. Mol Biol Cell 6:1781–1791.

Damsky CH, Werb Z (1992): Signal transduction by integrin receptors for extracellular matrix: Cooperative processing of extracellular information. Curr Opin Cell Biol 4:772–781.

Dejana ES, Colella S, Conforti G, Abbadini M, Gaboli M, Marchisio PC (1988): Fibronectin and vitronectin regulate the organization of their respective Arg-Gly-Asp adhesion receptors in cultured human endothelial cells. J Cell Biol 197:1215–1223.

Fath KR, Edhell CS, Burridge K (1989): The distribution of distinct integrins in focal contacts is determined by the substratum composition. J Cell Sci 92:67–75.

Geiger B, Salonon D (1992): A chimeric N-cadherin/β1-integrin receptor which localizes to both cell-cell and cell-matrix adhesions. J Cell Sci 103:943–951.

Gilbert JA, Banes AJ, Link GW, Jones GJ (1989): Surface strain of living cells in a mechanically active in vitro environment. In Dietrich, DE (ed): 'ANSYS Conference Proceedings.' Houston, TX: Swanson Analysis Systems.

Gilbert JA, Banes AJ, Link GW, Jones GL (1990): Video analysis of membrane strain: An application in cell stretching. Exp Tech 14:43–45.

Gilbert JA, Weinhold PS, Banes AJ, Link GW, Jones GL (1994): Strain profiles for circular cell culture plates containing flexible surfaces employed to mechanically deform cells in vitro. J Biomech 27:1169–1177.

Gimbrone MA, Cotran RS, Folkman J (1974): Human vascular endothelial cells in culture. J Cell Biol 60:673–684.

Girard P, Nerem RM (1995): Shear stress modulates endothelial cell morphology and F-actin organization through the regulation of focal adhesion-associated proteins. J Cell Physiol 163:179–193.

Hayman EG, Pierschbacher MD, Ohgren Y, Ruoslahte E (1983): Serum spreading factor (vitronectin) is present at the cell surface and in tissues. Proc Natl Acad Sci USA 80:4003–4007.

Hayman EG, Pierschbacher MD, Suzuki S, Ruoslahti E (1985): Vitronectin-a major cell attachment-promoting protein in fetal bovine serum. Exp Cell Res 160:245–258.

Herman IM (1987) Extracellular matrix-cytoskeletal interaction in vascular cells. Tissue Cell 19:1–19.

Horwitz A, Duggan E, Buck C, Beckerle MC, Burridge K (1986) Interaction of plasma membrane fibronectin receptor with talin-a transmembrane linkage. Nature 320:531–533.

Iba T, Sumpio BE (1991): Morphological response of human endothelial cells subjected to cyclic strain in vitro. Microvasc Res 42:245–254.

Ingber D (1991): Integrins as mechanochemical transducers. Curr Opin Cell Biol 3:841–848.

Ingber DE, Jamieson JD (1985): Cell as tensegrity structures: Architectural regulation of histodifferentiation by physical forces transduced over basement membrane. In Andersson LC, Gahmberg CG, Ekblom P (eds): 'Gene Expression During Normal and Malignant Differentiation.' Orlando, FL: Academic Press.

Kirchhofer D, Languino LR, Ruoslahti E, Pierschbacher MD (1990): $\alpha_2\beta_1$ integrins from different cell types show different binding specificities. J Biol Chem 265:615–618.

Kishimoto TK, O'Connor K, Lee A, Roberts TM, Springer TA (1987): Cloning of the β subunit of the leukocyte adhesion proteins: Homology to an extracellular matrix receptor defines a novel supergene family. Cell 48:681–690.

Kornberg L, Earp HS, Turner CE, Prockop C, Juliano RL (1991): Signal transduction by integrins: Increased protein tyrosine phosphorylation caused by clustering of β_1 integrins. Proc Natl Acad Sci USA 88:8392–8396.

Kornberg L, Earp HS, Parsons JT, Schaller M, Juliano RL (1992): Cell adhesion or integrin clustering increases phosphorylation of a focal adhesion-associated tyrosine kinase. J Biol Chem 267:23439–23442.

LaFlamme SE, Akiyama SK, Yamada KM (1992): Regulation of fibronectin receptor distribution. J Cell Biol 117: 437–447.

Li G, Mills I, Sumpio BE (1994): Cyclic strain stimulates endothelial cell proliferation: Characterization of strain requirements. Endothelium 22:177–181.

Lipfert L, Haimovich B, Schaller MD, Cobb BS, Parsons JT, Brugge JS (1992): Integrin-dependent phosphorylation and activation of the protein tyrosine kinase pp125^{FAK} in platelets. J Cell Biol 119:905–912.

Newman PJ, Kawai Y, Montgomery RR, Kunicki TJ (1986): Synthesis by cultured human umbilical vein endothelial cells of two proteins structurally and immunologically related to platelet membrane glycoproteins IIβ and IIIα. J Cell Biol 103:81–86.

Plow EF, Loftus JC, Levin EG, Fair DS, Dixon D, Forsyth J, Ginsberg MH (1986): Immunologic relationship between platelet membrane glycoprotein GPIIb/IIIa and cell surface molecules expressed by a variety of cells. Proc Natl Acad Sci USA 83:6002–6006.

Ruoslahti E, Pierschbacher MD (1987): New perspectives in cell adhesion: RGD and integrins. Science 238:491–497.

Schaller MD, Parson, JT (1994): Focal adhesion kinase and associated proteins. Curr Opin Cell Biol 6:705–710.

Sonnenberg A (1993): Integrins and their ligands. Curr Top Microbiol Immunol 184:7–35.

Sumpio BE, Banes AJ, Levin LG, Johnson G (1987) Mechanical stress stimulates aortic endothelial cells to proliferate. J Vasc Surg 6:252–256.

Thom D, Powell AJ, Rees DA (1979): Mechanisms of cellular adhesion IV. Role of serum glycoproteins in fibroblast spreading on glass. J Cell Sci 35:281–305.

Yano Y, Geibel J, Sumpio BE (1996a): Tyrosine phosphorylation of pp125^{FAK} and paxillin in aortic endothelial cells induced by mechanical strain. Am J Physiol 271:635–649.

Yano Y, Saito Y, Narumiya S, Sumpio, BE (1996b): Involvement of rho p21 in cyclic strain-induced tyrosine phosphorylation of focal adhesion kinase (pp125^{FAK}), morphological changes and migration of endothelial cells. Biochem Biophys Res Commun 224:508–515.