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Platelet-derived growth factor-BB modulates membrane mobility of β_1 integrins

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Abstract

Platelet-derived growth factor (PDGF)-BB elicits a migratory response including reorganization of the actin cytoskeleton in different cell types. Here we have investigated the effects of PDGF-BB stimulation on β_1 integrin containing focal adhesions in human diploid fibroblasts adhered to collagen type I. Stimulation with PDGF-BB dissociated focal adhesions and relocated β_1 integrins from focal adhesions to the periphery of the cells. These changes were rapid and transient in character. Relocation of β_1 integrins was prevented by inhibitors of phosphoinositide-3-kinase and protein kinase C. PDGF-BB stimulated fibroblasts exhibited an increased diffusion coefficient of cell surface β_1 integrins as determined by fluorescence recovery of photobleaching. The cell surface expression of β_1 integrins was not changed after stimulation with PDGF-BB. Our data suggest that PDGF-BB increases the dynamic properties of cell-surface β_1 integrins, which most likely are important for the migratory response elicited by PDGF-BB.

Keywords: Cell migration; Antibodies; Fluorescence recovery of photobleaching; Phosphoinositide-3-kinase; Focal adhesions; Fibroblasts; Collagen; Integrins; PDGF; Protein kinase C

Integrins are transmembrane heterodimeric glycoproteins, built from one α- and one β-subunit, which mediate cell–cell and cell–matrix adhesion [1]. Eight β- and 18-α integrin subunits have been characterized, which associate to form the more than 20 different integrin heterodimers found to date [1,2]. In cultured mesenchymally derived cells, integrins connect extracellular matrix fibers with the actin cytoskeleton at focal adhesion sites [3]. In addition to their role to physically link cells to the extracellular matrix these structures integrate and govern intracellular signaling events [4–6]. Focal adhesion assemblies include proteins such as vinculin, paxillin, and tensin. Talin and α-actinin, both of which interact directly with the integrin β_1 subunit [7,8], are also concentrated at the focal adhesion sites. In addition, focal adhesions contain several adaptor proteins and kinases involved in intracellular signaling, such as focal adhesion kinase (FAK), src family kinases, and growth factor receptors [4–6,9,10].

Platelet-derived growth factor-BB (PDGF-BB) [11] elicits a chemotactic response in different cell types to migrate through collagen type I-coated membranes [12–15]. The motility response elicited by PDGF-BB includes a rapid and transient change in the organization of actin-containing stress fibers resulting in membrane ruffling [14,16,17], accompanied by a redistribution of focal adhesion proteins from focal adhesions [18–20].

Here we investigated how β_1 integrins and focal adhesions are affected during short time PDGF-BB stimulation of fibroblasts cultured on collagen type I.

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Materials and methods

Reagents. Dulbecco's modified Eagle's medium (DMEM), MCDB 104 medium, normal horse and goat serum, trypsin-EDTA, penicillin, and streptomycin were from the National Veterinary Institute (Uppsala, Sweden). Fetal bovine serum (FBS) was either from Sera-Lab (Sussex, UK) or Integro BV (Zaandam, Holland). Bovine dermal collagen type I was from Cellon (Strassen, Luxembourg). PDGF-BB purified from supernatants of transfected yeast cells was donated by Dr. Carl-Henrik Heldin (Ludwig institute for Cancer Research, Uppsala, Sweden). Bovine serum albumin (fraction V) was from Boehringer Mannheim (Mannheim, Germany). Normal mouse, rat, and goat IgG, as well as FITC-labeled goat anti-rat IgG were from Sigma (St. Louis, MO). FITC-labeled goat anti-mouse IgG was from Becton–Dickinson (Bedford, MA). Biotinylated horse anti-mouse or goat anti-rabbit IgG and Avidin Texas Red were purchased from Vector (Burlingame, CA), Bodipy Phallacidin was from Molecular Probes (Eugene, OR), and Fluoromount G was from Southern Biotechnology Associates (Birmingham, AL).

Antibodies. The TS2/16 mouse hybridoma producing monoclonal anti-β_1 integrin IgG [21] was donated by Dr. Martin Hemler (Harvard Medical School, Boston, USA). TS2/16 IgG from hybridoma supernatants was purified on protein G–Sepharose from hybridoma supernatants (Pharmacia Biotech, Uppsala, Sweden). Mouse monoclonal anti-β_1 integrin antibodies K20 and P4C10 were from Immunotech (Marseille, France) and Gibco-BRL LifeTechnologies (Gaithersburg, MD), respectively. Mouse monoclonal anti-β_1 integrin antibody LM534, mouse monoclonal anti-α_2 integrin antibody P1E6, and rabbit polyclonal anti-α_2 integrin IgG were from Chemicon International (Temecula, CA). Rat monoclonal anti-β_1 integrin antibody 9EG7 was from Pharmingen (San Diego, CA). Monovalent Fab fragments from TS2/16 IgG were prepared by digestion of purified IgG with papain (Sigma, St. Louis, MO) according to standard protocols. After digestion, Fab fragments were purified by molecular sieve chromatography and Fc fragments were removed by affinity chromatography on protein G–Sepharose.

Cells and cell culture. AG1518 human foreskin fibroblasts, purchased from Human Genetic Mutant Cell Repository (Camden, NJ), were grown in DMEM supplemented with 10% FBS, 50 μg/ml streptomycin, and 60 μg/ml penicillin, and were used between passages 17 and 22 (passage split 1:2). MCDB 104 medium with the addition of 0.1% BSA was used for serum-free culture of AG1518.

Immunofluorescence. Coverslips were coated with 50 μg/ml collagen type I in PBS at 4 °C for more than 12 h and then washed three times with PBS. AG1518 fibroblasts were trypsinized, washed two times, suspended in serum-free MCDB 104 medium, and allowed to spread on the coverslips for 3 h. Cells were treated with 0, 5 or 25 ng/ml PDGF-BB for the desired time periods. The coverslips were gently washed once with cold PBS and cells were fixed for 10 min with cold 2% paraformaldehyde in PBS, permeabilized for 15 min with cold 0.5% Triton X-100 in PBS, and blocked with 0.1 M glycine, and 0.5% BSA in PBS at 4 °C for more than 12 h. Coverslips were incubated for 1 h with primary antibody together with 5% normal horse (for mouse IgG) or goat (for rabbit IgG) serum, 30 min with biotinylated secondary antibody, 30 min with avidin Texas Red together with Bodipy Phallacidin, and finally mounted on ethanol-washed glass slides in Fluoromount G. To score cells for the presence or absence of focal adhesion staining or peripheral staining, 50 cells from each coverslip were observed. Cells were scored as being focal adhesion-positive if more than 10 clear focal adhesions could be seen and scored as having peripheral staining if close to or more than half the cell periphery was outlined.

Interference reflection microscopy. Coverslips were precoated with 30 μg/ml collagen type I. AG1518 fibroblasts were seeded onto the coverslips in serum-free MCDB 104 medium was with 0.2% BSA and incubated for 2–3 h in a cell culture incubator to allow cell spreading. The coverslips were placed in a holder kept at 37 °C and warm medium added. Cells were observed and photographed using a Zeiss Axiovert 35 inverted microscope with a plan Neofluar 63x/1.25 objective and equipped for interference reflection and phase contrast microscopy. Images were taken both at the phase contrast and interference reflection modes at each time point. Images of the same individual cells were recorded both before and after incubations in the presence of 20 ng/ml PDGF-BB for 15 and 45 min.

Fluorescence recovery after photobleaching. Preparation of cells: FITC was covalently conjugated to monovalent TS2/16 Fab fragments using Fluorescein-5-EX succinimidyl ester (Molecular Probes, Eugene, OR) according to the recommendations of the manufacturer. The FITC-labeled Fab fragments specifically precipitated the expected β_1 integrins from surface-labeled AG1518 fibroblasts (data not shown). AG1518 fibroblasts were seeded directly onto 12 mm circular coverslips in DMEM containing 10% FBS and allowed to spread in a cell culture incubator for about 20 h. Cells were then incubated for 20 min in room temperature with 70 μg/ml FITC-coupled monovalent TS2/16 Fab fragments diluted in MCDB 104 medium containing 0.1% BSA. After labeling, cells were washed gently 3 times with the same medium and mounted by placing the coverslip with cells on a glass slide with fitted spacers made from Parafilm separation paper, to create an approximately 50 μl incubation chamber. A drop of MCDB 104 containing 0.1% BSA and with or without 25 ng/ml PDGF-BB was added to the coverslip, and then a square coverslip was put on top. The chamber was sealed with a hot mixture of wax and vaseline 1:1. The photobleaching experiments were performed at 37 °C within 60 min after addition of medium to the wet chamber.

The lateral diffusion of β_1 integrins was measured by fluorescence recovery after photobleaching (FRAP) as described elsewhere [22–26]. In short, a 488 nm laser beam (Argon laser, Type 2020-3, Spectra Physics, Mountain View, CA) run in current mode at 35 Amps, focused with a Zeiss 63X oil-immersion objective (NA = 1.4) to a spot with an estimated $1/e^2$ radius (w) of 0.89 μm, was used to excite the fluorescent Fab fragments and to bleach the fluorescence during 500 ms. The fluorescence from the illuminated spot was quantified with a photomultiplier tube (SD Photometer, Carl Zeiss, Oberkocken, Germany) at gradually increasing intervals usually from 1 to 40 s, and the final point was taken after 120 s. The control of the laser beam and recording of the fluorescence recovery were done by a computer. The diffusion coefficient, D, was calculated from the half-time for recovery ($\tau_{1/2}$ = the time required to obtain 50% of final recovery) by the equation $D = w^2\gamma/4t_{1/2}$ The parameter γ depends on the extent of photobleaching [22]. D is given as $cm^2\,s^{-1}$. The mobile fraction, R, of integrins was calculated from the formula $(F_\infty - F_0)/(F_i - F_0)$, where F_i is the initial fluorescence intensity before bleaching, F_0 is the fluorescence intensity immediately after bleaching, and F_∞ is the recovered fluorescence intensity after 120 s, and R is given as percentage.

Flow cytometry. According to protocol one, AG1518 fibroblasts in 60-mm dishes (Falcon, Becton–Dickinson, Plymouth, UK) were incubated in serum-free MCDB 104 medium for 24 h. Cells were then stimulated with 12.5 or 50 ng/ml PDGF-BB for 1 or 3 h, or left untreated as controls. After trypsinization, the cells were incubated in suspension for 1 h at 4 °C with 2.5 μg/ml mAb TS2/16, mAb P4C10, or normal mouse IgG in PBS supplemented with 0.5% BSA and 10 μg/ml normal goat IgG, followed by FITC-labeled goat anti-mouse IgG for 0.5 h at 4 °C. According to protocol two, AG1518 fibroblasts were trypsinized, counted, and incubated in HBS (20 mM Hepes, 150 mM NaCl, pH 7.4) without additions, with 25 ng/ml PDGF-BB or with 5 mM $MnCl_2$, for 20 min at 37 °C. The cells were then incubated in suspension for 1 h at 4 °C with 10 μg/ml mAb TS2/16, normal mouse IgG, mAb 9EG7, or normal rat IgG in HBS supplemented with 0.5% BSA, 10 μg/ml normal goat IgG, and the same addition of PDGF-BB or $MnCl_2$ as before. This was followed by FITC-labeled goat anti-mouse or goat anti-rat IgG for 0.5 h at 4 °C. Fluorescence was

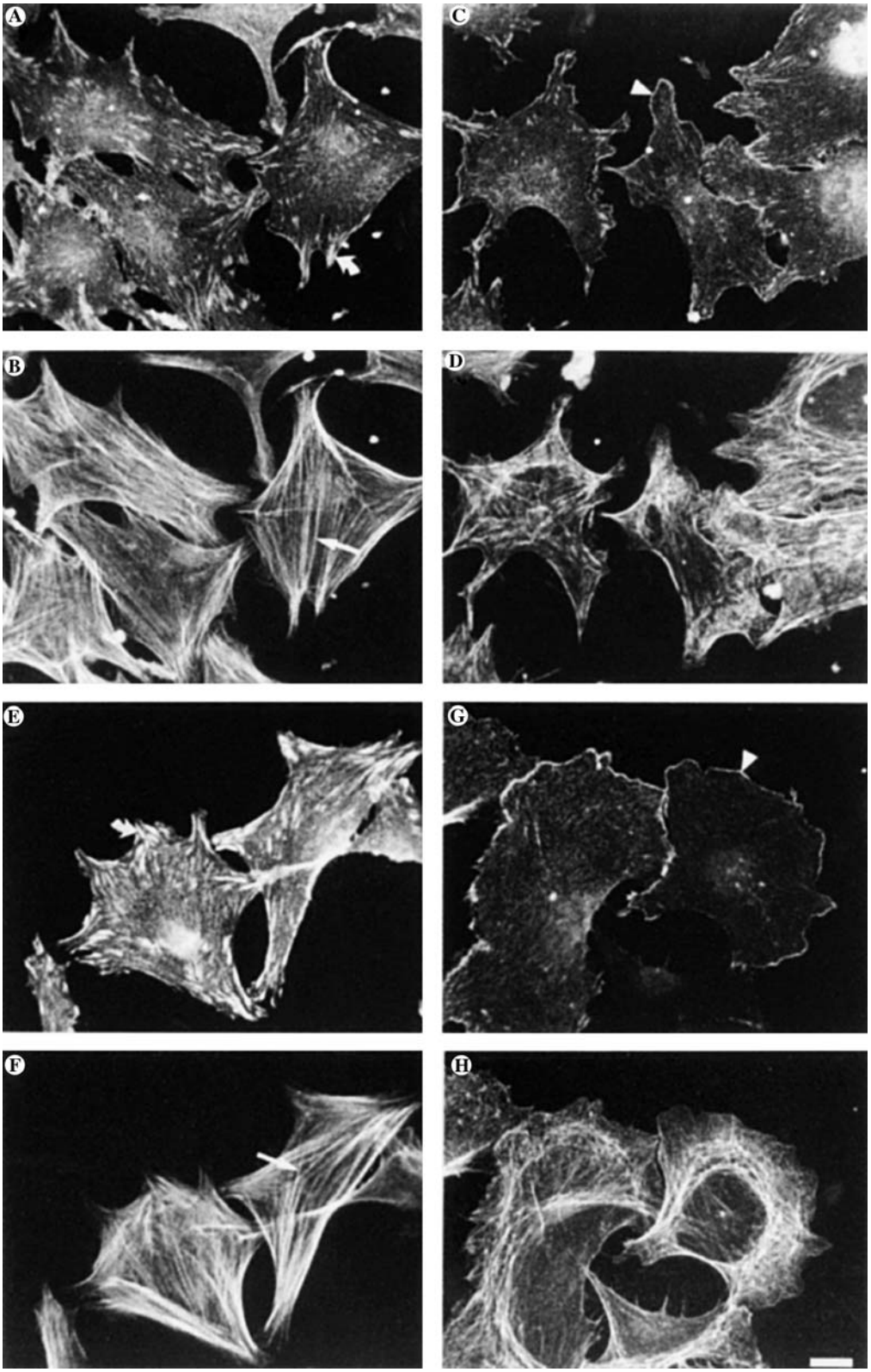

Fig. 1. Illustration of integrin staining in focal adhesions and around the cell periphery. AG1518 fibroblasts on collagen-coated coverslips were stained for β_1-integrins with mAb K20 (A,C), α_2-integrin with mAb P1E6 (E,G), and actin filaments (B, D, F, and H) as described in Materials and methods. Cells were stimulated with 25 ng/ml PDGF-BB for 15 min (C, D, G, and H) or left as controls (A, B, E, and F). The unstimulated cells in A, B, E, and F show β_1- (bent arrow in A) and α_2- (bent arrow in E) integrin staining in focal adhesions as well as actin stress fibers (arrows in B and F). Arrowheads show peripheral β_1- (in C) and α_2- (in G) integrin staining in response to PDGF-BB stimulation. Bar in H represents 20 μm.

analyzed on a Becton–Dickinson FACScan with CellQuest software (Becton–Dickinson, Bedford, MA).

Binding of TS2/16 IgG to cultured fibroblasts. TS2/16 IgG was iodinated using ^{125}I and iodobeads (Pierce Chemicals, Rockford, IL). AG1518 fibroblasts were seeded into the wells of 24-well plates that had previously been coated with DMEM containing 10% FBS. The cells were incubated in MCDB 104 medium supplemented with 0.1% BSA overnight and then stimulated for the desired time periods with 25 ng/ml PDGF-BB at 37 °C. The cells were then washed with ice-cold buffer (0.1% BSA, 0.14 M NaCl, 4.7 mM KCl, 0.65 mM $MgSO_4$, 1.22 mM $CaCl_2$, and 10 mM Hepes, pH 7.4) and incubated on a rocker in a cold room for 40 min with 10 nM [^{125}I]TS2/16 IgG and non-labeled TS2/16 IgG between 0 and 2.0 μM diluted in the same buffer. The cells were then washed again and lysed with 1 M NaOH.

Results

PDGF-BB stimulation leads to a transient relocation of β_1 integrins from focal adhesions

Over 95% of AG1518 fibroblasts that had adhered and spread on collagen-coated coverslips for 2–3 h displayed prominent β_1 integrin containing focal adhesions (Fig. 1A), as well as prominent stress fiber arrays (Fig. 1B). Stimulation with PDGF-BB induced a transient decrease in the number of cells that displayed β_1 integrin containing focal adhesions. Stimulation with 25 ng/ml PDGF-BB 15 min reduced the number of clear β_1 integrin-containing focal adhesions in a majority of the cells (Fig. 1C). Stimulation with 12.5 ng/ml PDGF-BB had a similar, but less marked, effect (data not shown). At the same time as staining in focal adhesions decreased, β_1 integrin staining instead became visible in a rim around the cell periphery. This peripheral staining was seen as a continuous line along parts of, or the whole cell circumference (Fig. 1C). After stimulation of AG1518 fibroblasts for 15 min with 25 ng/ml PDGF-BB, 53% of the cells displayed peripheral β_1 integrin staining (Fig. 2A). This pattern of β_1 integrin staining was gradually replaced by small but clearly visible focal adhesions or contacts concentrated towards the cell periphery (data not shown). The decrease in the number of cells that displayed β_1 integrin containing focal adhesions upon PDGF stimulation was also apparent in AG1518 fibroblasts that had been cultured overnight in serum-free medium on collagen-coated coverslips (data not shown).

After a 2–3 h culture period on collagen type I-coated coverslips AG1518 fibroblasts exhibited α_2-, but not α_1- or α_3-, chain, in focal adhesions. The α_2-chain was in a similar fashion as the β_1-chain relocated from focal adhesions to the cell periphery upon PDGF-stimulation (Figs. 1E and G, and Fig. 2B). The focal adhesion proteins vinculin, talin, and paxillin were transiently removed from focal adhesions and displayed at the cell periphery after PDGF-BB stimulation (data not shown).

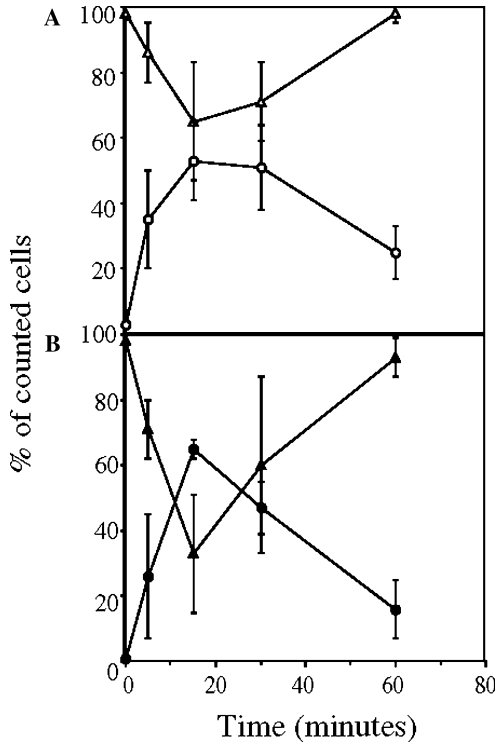

Fig. 2. Effects of PDGF-BB on integrin distribution. AG1518 fibroblasts were allowed to spread on collagen-coated coverslips for a total of 3 h. During the last 5, 15, 30 or 60 min the cells were incubated with 25 ng/ml PDGF-BB. Cells were stained for β_1-integrin (monoclonal antibodies K20 or LM534), α_2-integrin (AB1936 or monoclonal antibody P1E6) and actin filaments as described in Materials and methods. The cells were then scored for the presence of β_1- or α_2-integrin-containing focal adhesions and for the presence of peripheral β_1- or α_2-integrin staining. Averages from three independent experiments ± 1 SD are shown. (A) Percentage of cells with β_1-integrin staining in focal adhesions (empty triangles) and percentage of cells with peripheral β_1-integrin stain (empty circles). (B) Percentage of cells with α_2-integrin staining in focal adhesions (filled triangles) and percentage of cells with peripheral α_2-integrin stain (filled circles).

PDGF-BB stimulation leads to dissociation of focal adhesions

A total of 10 AG1518 fibroblasts were analyzed individually by interference reflection microscopy (IRM) before and after addition of PDGF-BB, and the number of focal adhesions on each cell was counted. On average, the cells had 30 ± 17 focal adhesions. After 15 min of incubation with 20 ng/ml PDGF-BB only 37% of these focal adhesions remained ($p < 0.05$), while the rest had disappeared or translocated (Table 1). Also, a majority of cells acquired peripheral contacts comparable to the peripheral integrin staining seen with immunofluorescence (Table 1).

Relocation of β_1 integrins depends on phosphoinositide-3-kinase and protein kinase C

Incubation of cells with the phosphoinositide-3-kinase (PI3K) inhibitor LY294002, or the protein kinase C

Table 1
Effects of PDGF-BB stimulation on focal adhesions analyzed by interference reflection microscopy

Cell	No. of focal contacts			Peripheral contacts	
	Before PDGF	Remaining after PDGF	% remaining	Before PDGF	After PDGF
1	7	0	0	+/–	+
2	50	30	60	–	–
3	62	47	76	–	+/–
4	26	16	62	–	+
5	8	1	12	–	+
6	38	0	0	+/–	++
7	26	7	27	+/–	+
8	36	21	58	–	++
9	24	14	58	–	+/–
10	25	5	20	–	+
Average:	30 ± 17	14 ± 15	37 ± 28		

AG1518 fibroblasts were allowed to spread on collagen-coated coverslips for a total of 2–3 h. Glass coverslips with adhered cells were mounted as described in Materials and Methods. Images of the same individual cells were recorded both before and after incubation with 20 ng/ml PDGF-BB for 15 min. The number of focal contacts on each cell was counted from the images.

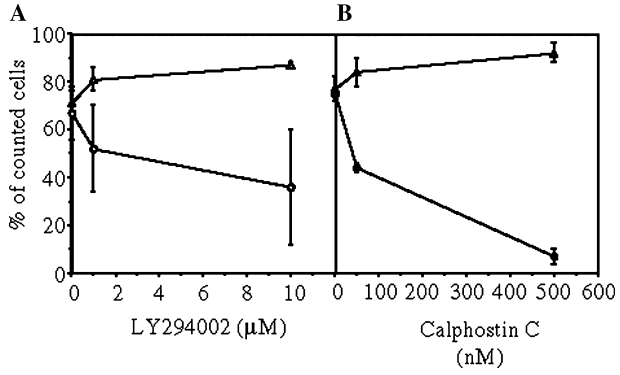

Fig. 3. Effects of inhibitors of phosphatidylinositol 3-kinase and protein kinase C on the PDGF-BB-induced integrin redistribution. AG1518 fibroblasts were allowed to spread on collagen-coated coverslips for a total of 3 h and then further incubated for 15 min with 25 ng/ml PDGF-BB together with LY294002 or calphostin C at the indicated concentrations. Cells were stained for β_1-integrin and actin filaments as described in Materials and methods. The presence of β_1-integrin containing focal adhesions and peripheral β_1-integrin staining were then scored manually. Averages from two independent experiments ± total spreading are shown. (A) LY294002-inhibition of relocation of β_1-integrins. Percentage of cells with β_1-integrin staining in focal adhesions (triangles) and percentage of cells with peripheral β_1-integrin stain (circles). (B) shows calphostin C inhibition of relocation of β_1-integrins. Percentage of cells with β_1-integrin staining in focal adhesions (triangles) and percentage of cells with peripheral β_1-integrin stain (circles).

inhibitor calphostin C, inhibited the transient relocation of β_1 integrins and the actin ruffling induced by PDGF-BB (Fig. 3). The protein kinase A inhibitor KT5720 was without effect on integrin distribution and actin reorganization (data not shown).

PDGF-BB stimulation leads to increased lateral mobility of β_1 integrins

In order to examine the effects of PDGF-BB on the interaction between β_1 integrins and the cytoskeleton we performed FRAP which measures the lateral mobility of β_1 integrins in the plane of the cell membrane. Cells were incubated with FITC conjugated monovalent TS2/16 Fab fragments and then incubated in the presence or absence of 25 ng/ml PDGF-BB at 37 °C for different time periods. Measurements were not performed at sites close to visible focal adhesions. Compiled data from several measurements are shown in Fig. 4. The average diffusion coefficient of β_1 integrins in non-stimulated AG1518 fibroblasts was $5.1 \pm 2.0 \times 10^{-10}\,\mathrm{cm^2\,s^{-1}}$ (Fig. 4B), with a mobile fraction of $67 \pm 21\%$ ($n = 18$).

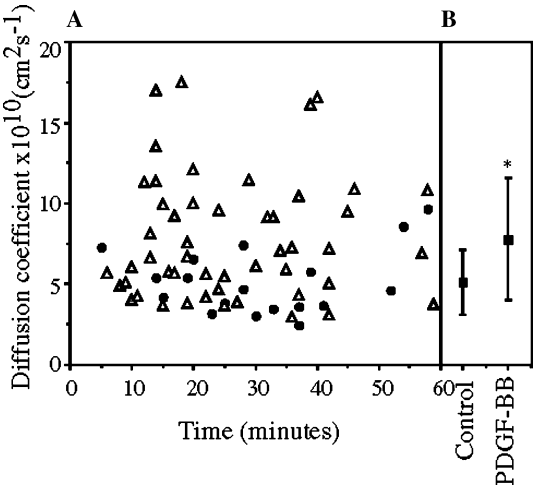

Fig. 4. Effects of PDGF-BB on membrane mobility characteristics of β_1 integrins. AG1518 fibroblasts were grown overnight on coverslips as described in Materials and methods. The cells were incubated with FITC-coupled TS2/16 Fab fragments and then stimulated with 25 ng/ml PDGF-BB for up to 60 min or left as controls. The scatter plot in (A) shows the values of the diffusion coefficient in individual PDGF-BB stimulated (empty triangles) and control (filled circles) cells. (B) shows the average diffusion coefficient ± 1 SD in PDGF-BB stimulated ($n = 50$) and control ($n = 18$) cells. The difference in average diffusion coefficient between stimulated and control cells was significant ($p < 0.01$ (*)) when tested with Student's t test.

AG1518 fibroblasts stimulated with 25 ng/ml PDGF-BB between 1 and 60 min had an increased average diffusion coefficient, $7.8 \pm 3.8 \times 10^{-10}\,\mathrm{cm^2\,s^{-1}}$ (Fig. 4B), whereas the mobile fraction was only marginally affected at $61 \pm 21\%$ ($n = 50$).

Cell surface expression of β_1 integrins is not changed after short time PDGF-BB stimulation

Adherent AG1518 fibroblasts were stimulated with 12.5 or 50 ng/ml PDGF-BB for 3 h (Fig. 5) and then trypsinized and stained with anti-β_1 integrin mAbs TS2/16 and P4C10, or anti-α_2 integrin mAb P1E6 followed by analyses by flow cytometry. Mean fluorescence intensity did not change after 3 h of PDGF-BB stimulation. In a second series of experiments we looked for more rapid changes that may be related to conformational changes in the integrins. Here, we trypsinized AG1518 fibroblasts and immediately after counting the cells stimulated them with 25 ng/ml PDGF-BB for 20 min. The presence of PDGF β-receptors on the cell surface after trypsinization was verified by immunoprecipitation and immunoblotting for the receptor protein, and for tyrosine phosphorylated receptor in the PDGF BB-stimulated samples (data not shown). After stimulation, the cells were stained with the anti-β_1 integrin mAbs TS2/16 and 9EG7 (Fig. 6). The mean fluorescence intensities on cells stained with TS2/16 did not change significantly after stimulation with PDGF-BB. The 9EG7 epitope can be induced by Mn^{2+} and the binding of 9EG7 increased in response to incubation with 5 mM Mn^{2+}. Binding of 9EG7 decreased, however, after stimulation with PDGF-BB although this effect was less pronounced (Fig. 6). Binding of TS2/16 IgG to

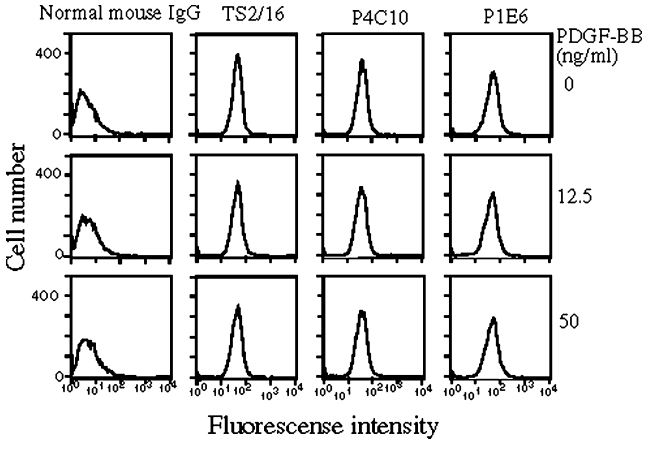

Fig. 5. Effects of PDGF-BB on cell surface expression of β_1 integrins. Serum-starved AG1518 fibroblasts cultured on collagen-coated dishes were stimulated with 12.5 or 50 ng/ml PDGF-BB for 3 h or left untreated as controls. The cells were stained with anti-β_1 integrin mAbs TS2/16 and P4C10, anti-α_2 integrin mAb P1E6, or normal mouse IgG and subjected to flow cytometry all as described in Materials and methods.

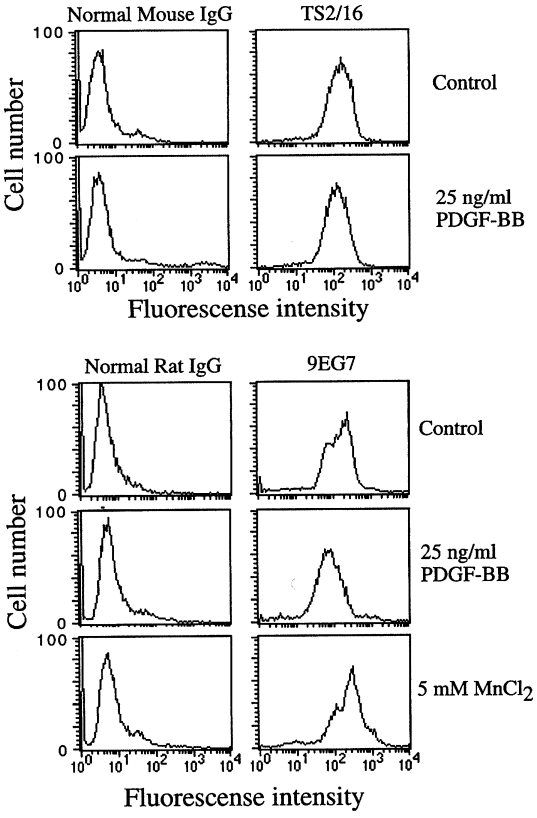

Fig. 6. Effects of PDGF-BB on cell surface expression of β_1 integrins. Trypsinized and suspended AG1518 fibroblasts were incubated in the presence or absence of 25 ng/ml PDGF-BB for 20 min. After stimulation, the cells were stained with the anti-β_1 integrin mAbs TS2/16 and 9EG7 and subjected to flow cytometry all as described in Materials and methods.

adherent AG1518 fibroblasts was further investigated by Scatchard analysis. The resulting Scatchard plots revealed no differences in antibody binding between PDGF BB-stimulated and control cells (data not shown). The number of binding sites for TS2/16 IgG was approximately 4×10^6 per cell and the estimated K_d:'s were around 0.13 μM.

Discussion

We report that $\alpha_2\beta_1$ integrins in human diploid fibroblasts cultured on collagen type I rapidly and transiently relocated from focal adhesions to the cell periphery after stimulation with PDGF-BB. This redistribution coincided in time with a reduction in the number of focal adhesions that could be visualized by IRM and with a loss of stress fiber arrays. PDGF-BB also induced an increased lateral mobility of β_1 integrins in the cell membrane as detected by FRAP but did not induce any change in the apparent cell surface expression of β_1 integrins. These effects occurred within one hour after addition of PDGF-BB. When taken together these data suggest that stimulation of fibroblasts with

PDGF-BB increases the dynamic turnover of collagen-binding β_1 integrins.

It is known that activation of PI3K in rat embryonic fibroblasts by stimulation of PDGF-BB leads to a restructuring of focal adhesion sites [20]. Furthermore, PI3K activity is essential for PDGF β-receptor elicited chemotaxis and actin reorganization in pig aortic endothelial cells [27] and for the stimulation of fibroblast-mediated collagen gel contraction by PDGF-BB [28,29]. Kundra et al. [30] demonstrated that PDGF β-receptor induced cell migration depends on a balance of PI3-K and PLC-γ activities, which promote migration, and Ras-GAP, which suppresses migration. Our present data showing that the PI3K inhibitor LY294002 inhibits redistribution of β_1 integrins in response to PDGF-BB stimulation are thus in agreement with these earlier reports.

In the present study, the anti-β_1 integrin monoclonal antibody TS2/16 [21] was used in the FRAP experiments. TS2/16 IgG or monovalent Fab fragments increase the apparent affinity of isolated $\alpha_5\beta_1$ and $\alpha_2\beta_1$ integrins for their respective ligands, as well as stimulate cell adhesion to extracellular matrix proteins that are mediated by β_1 integrins [31]. In K562 human erythroleukemia cells, TS2/16 Fab fragments bind to approximately 90% of the reported number of $\alpha_5\beta_1$ fibronectin receptors, which are the only β_1 integrins present on these cells [32–34]. Based on these data, it can be assumed that TS2/16 Fab fragments detect a majority, and not only a subpopulation, of the β_1 integrins. The binding of TS2/16, the function blocking antibody P4C10 or the anti-$\alpha_2\beta_1$ antibody P1E6 was unaffected by stimulation with PDGF-BB for up to 3 h. These data show that PDGF-BB stimulation does not induce changes in cell surface expression of β_1 integrins. Binding of mAb 9EG7 to β_1 integrins is increased in response to Mn^{2+} and ligands for β_1 integrins [35] and has been suggested to recognize an 'activated' conformation of β_1 integrins [36]. The decrease in binding of 9EG7 after PDGF-BB stimulation is compatible with the fact that the fraction of un-ligated β_1 integrins increased as a result of the release of β_1 integrins from focal adhesions.

Usually, a reduction in the size of a membrane protein complex results in a more rapid lateral diffusion, i.e., a higher value of the diffusion coefficient [37]. The *D*-value is thus sensitive to altered oligomerization and/or ligand binding [38]. It could also reflect a less frequent interaction and arrest at lipid rafts and other domains in the membrane [39]. The mobile fraction (*R*, %) of receptors reflects whether the receptors are free to move, or, for instance, are hooked up by interactions with the cytoskeleton or membrane domains at any given moment [38,39]. In the present study, *D* was increased for the β_1 integrins after PDGF-BB stimulation, while *R* remained unaffected. This speaks for an increased rate of mobility of smaller β_1 integrin- or β_1 integrin-associated complexes with similar retention in putative membrane domains.

Small non-ligand bound β_1 integrin complexes on the dorsal surface of the lamellopodia of moving 3T3 cells undergo rapid transport to the leading edge separated by periods of random diffusion, whereas clustering or ligand binding of β_1-integrins couples integrins to the cytoskeleton and leads to a steady rearward transport [40,41]. Focal adhesions, visualized by green fluorescent protein (GFP)-tagged β_1 integrins, are motile in stationary 3T3 fibroblasts and are continuously moving toward the cell center [42]. Furthermore, GFP-tagged β_3 integrins move rapidly within an individual focal contact in bone marrow-derived endothelial cells [43]. The $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins are also dynamically transported by a process involving rab-protein-dependent vesicular transport in 3T3 fibroblasts [44]. Stimulation of 3T3 cells with PDGF-BB resulted in a rab4-dependent recycling of $\alpha_v\beta_3$ integrin from early endosomes to the plasma membrane where they potentially can form new focal contacts [44]. Collectively, these reports demonstrate that integrins are dynamic both within and between focal contact/adhesion sites. An increased turnover is likely to facilitate cell spreading and cell migration. Our present results suggest that PDGF-BB facilitates migration and spreading on collagenous supports at least partly by increasing the turnover of collagen-binding β_1 integrins.

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