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ARTICLE

Tyrosine Phosphorylation of a ~30 kD Protein Precedes αvβ3 Integrin-signaled Endothelial Cell Spreading and Motility on Matrix Proteins

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A microvascular endothelial cell line (CD clone 4) isolated from murine lung adheres to and spreads well on fibronectin, vitronectin, and fibrinogen, but poorly on collagen type IV and laminin. Ligating cell surface αv, β3, α4, α5, or β1 integrin receptors with monospecific antibodies promoted a dramatic cell spreading and motility on vitronectin or collagen IV. Antibodies directed to other adhesion molecules, including αIIb, PECAM-1, and P-selectin were ineffective. Ligation with monoclonal anti-αv or -β3, but not -α4, -α5, or -β1 antibodies, induced a rapid, and dose-dependent tyrosine phosphorylation of a ~30 kD protein, which preceded CD clone 4 endothelial cell spreading and motility and was partially inhibited by genistein and completely inhibited by BAPTA. All other antibodies tested did not induce the tyrosine phosphorylation of the 30 kD protein as well as cell spreading and motility. The present results suggest that β1 and β3 integrins employ different biochemical mechanisms in signaling endothelial cell spreading and motility and that the tyrosine phosphorylation of the 30 kD protein (and probably other proteins) may play an important role in signaling β3 integrin-mediated endothelial cell interaction with other cells (e.g., tumor cells) and extracellular matrix. (Pathology Oncology Research Vol 2, No1–2, 21–29, 1996)

Key words: integrin; motility; spreading; tyrosin phosphorylation; extracellular matrix

Introduction

Vascular endothelial cells express six members of the β1 integrin subfamily (i.e., α1-6/β1), three αv-coupled heterodimers (i.e., αvβ1, αvβ3, αvβ5), and α6β4.[1-4] These integrin receptors, together with other adhesion molecules such as cadherins and PECAM-1 (platelet endothelial cell adhesion molecule-1), play an essential role in establishing endothelial cell – endothelial cell adhesion, maintaining the physical integrity of endothelial cell monolayers, and mediating endothelial cell – matrix interactions.[4,5] Some of these integrin receptors (e.g., αvβ3) also are involved in heterologous cell-cell interactions such as tumor cell-endothelial cell, leukocyte – endothelial cell, and platelet – endothelial cell adhesion.[3,6-9] More recently, vascular integrins have been demonstrated to transduce a diversity of biological, biochemical, and biomechanical signals across the endothelial cell membrane, among which are calcium transients, cytoplasmic alkalinization, protein tyrosine phosphorylation, and cytoskeletal rearrangements.[10-12] These integrin-transmitted signals, in coordination with signals delivered from various soluble growth factors, motility factors, and cytokines, modulate phenotypic properties of vascular endothelial cells including adhesion, spreading, migration/motility, proliferation, apoptosis, and differentiation angiogenesis, etc.[13-17]

Occupation of integrins by their ligands or clustering the integrin receptors with antibodies (Abs) has previously been shown to induce tyrosine phosphorylation of some proteins including, among others, pp125[FAK],[18,19] paxillin,[20] and tensin.[21] Using a mouse pulmonary microvasculature-derived

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Abbreviations: Ab: antibody; BAPTA-AM: bis(2-aminophenoxy)-ethane-N, N, N', N'-tetraacetate, acetoxymethyl ester; DMEM: Dulbecco's Minimum Essential Medium; FAK: focal adhesion kinase; mAb: monoclonal antibody; pAb: polyclonal antibody; PKC: protein kinase C; SEM: subendothelial matrix; Vn: vitronectin.

endothelial cell line (i.e., CD clone 4), we report here a ~30 kD protein which is tyrosine-phosphorylated following ligation of integrin αv or β3 with monospecific antibodies. The tyrosine phosphorylation of the 30 kD protein temporally precedes the antibody-induced endothelial cell spreading and motility on matrix proteins.

Materials and Methods

Cell culture

CD clone 4 endothelial cells were cloned from pooled mouse lung microvasculatures and characterized and cultured as previously detailed.[22] Cells were routinely cultured in DMEM supplemented with 10% FBS, 0.01% gelatin, and various antibiotics in a humidified atmosphere with 5% CO_2.

Cell plating on matrix proteins and subendothelial matrix (SEM)

Coating of coverslips with various matrix proteins (i.e., vitronectin, fibronectin, laminin, type IV collagen, and fibrinogen), preparation of intact SEM, and endothelial cell plating were performed essentially as described previously.[4,7]

Antibody ligation and Western blotting of tyrosine phosphorylated proteins

Half a million of CD clone 4 cells were plated onto coated 6-well culture dishes (2.5 x 10^5/well) and incubated for various time periods in serum-free DMEM. Thereafter cells were incubated at 37°C with different concentrations of Abs (*Table 1*) for various intervals as indicated in the Results. In some experiments cells were further incubated with a secondary Ab. In another set of experiments, cells were pretreated with genistein or BAPTA-AM [bis (2-aminophenoxy)-ethane-N, N, N', N'-tetraacetate, acetoxymethyl ester] after initial cell adhesion and before Ab ligation. The cell morphology was monitored using phase contrast microscopy. For immunoblotting, after extensive washing following Ab treatment cells were lysed in situ with 1 ml of boiling lysis buffer (10 mM Tris-HCl, pH 7.4, 1% SDS) and aliquots taken for protein concentration measurement using the Lowry method (Bio-Rad). Equal amounts of proteins were separated on denaturing 12% SDS-PAGE under reducing conditions. Following transfer, blots were incubated with either monochonel PY 20-derived RC20H anti-phosphotyrosine Ab conjugated to HRP (Transduction Laboratories, Ky) or monoclonal anti-phosphotyrosine Ab 4G10 (Upstate Biotech). The tyrosine phosphorylated proteins were then detected with ECL (Amersham). To control protein loading, the membrane blot was stripped and reprobed with a monoclonal antibody (mAb) to myosin light chain (MLC). In some experiments, the blot was reprobed with a mAb to focal adhesion kinase (FAK; Upstate Biotech).

Phagokinetic track motility assay

CD clone 4 cell motility was assessed using the method described previously.[23] Briefly, glass coverslips coated with vitronectin (1 μg/ml) or collagen IV (25 μg/ml) were coated with a layer of gold particles. Then CD clone 4 cells (50,000 cells/18 mm^2 coverslip) were plated in serum-free DMEM for 1 h after which different concentrations of various Abs were directly added to the medium. Cell motility was monitored on a phase contrast microscope (dark field) as the area cleared by motile cells. The degree of cell motility was semi-quantitated as described in Results.

***Table 1.* Summary of antibodies and their effects**

Ab against	*Nature of Ab*[a]	*Source*[b]	*Spreading/Motility*[c]	*~30kD protein phosphorylation*[d]
αvβ3	pAb	Gibco (12119-012)	++/+	–
α5β1	pAb	Gibco (12118-014)	++/+	–
αv	mAb	Gibco (VNR147;12084-018)	++++/++	+
β3	mAb (OPG-2)	dr. T. Kuniczki[7,8]	++++/++	+
α5	mAb	Oncogene (CP12)	++/+	–
β1	mAb	Gibco (P4C10;12086-013)	+++/++	–
α4	mAb	Gibco (P4C2;12077-012)	+++/+	–
P-selectin	mAb	Accurate (MAS-543P)	–	–
PECAM-1	mAb 1.3	dr. P. Newman[22]	–	–
αIIb	mAb (AP4)	dr. T. Kuniczki	–	–
MOPC 21	mAb (IgG1)	Sigma	–	–
IgG	pAb	ICN	–	–

[a]pAb – polyclonal antibody (rabbit); mAb – monoclonal antibody (mouse)
[b]Specificity of these antibodies has been characterized.
[c]For spreading assay CD4 clone 4 cells were plated onto vitronectin for 15 min followed by incubation with mAbs (5 μg/ml) or pAb (10 μg/ml) for 15 min. At the end of the experiment, cells were fixed in 4% paraformaldehyde and 400 cells were assessed for spreading. The relative percentage of spread cells, i.e. minimal spreading (<10%), 50-75% spreading, 75-95% spreading, and >95% spreading, was arbitrarily assigned with -, ++, +++, and ++++, respectively. For motility assays, 50000 CD clone 4 cells were plated on collagen IV or Vn coated coverslips. The extent of cell motility was evaluated by the size of cleared areas and number of migrating cells.[23] The signs of -, +, and ++ indicate minimal motility (as observed with MOPC control), moderate motility (50-80% of cells migrated with easily identifiable cleared zones), and high motility (>90% cells migrated with prominent cleared zones).
[d]CD clone 4 cells were seeded onto Vn (1 μg/ml) for 15 min followed by incubation with either pAbs (10 μg/ml) or mAbs (5 μg/ml) for 15 min. Cell lysates were then prepared and immunoblotting performed with anti-phosphotyrosine Ab RC20H.

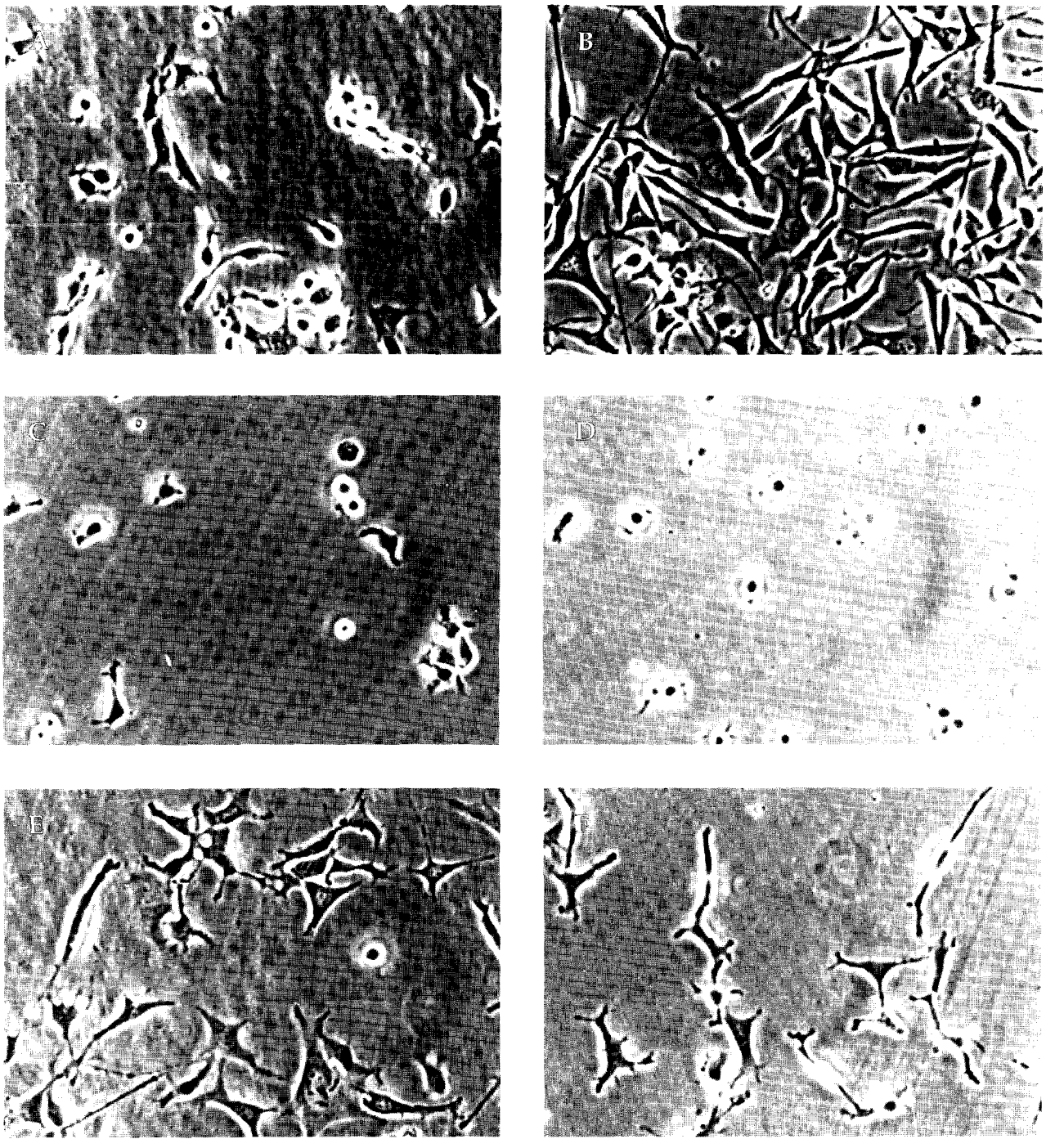


Figure 1. *Behavior of CD clone 4 microvascular endothelial cells on different substrates. 2.5 x 10⁵ CD clone 4 cells were seeded onto coverslips coated with BSA (A, 100 µg/ml), fibronectin (B, 25 µg/ml), collagen type IV (C, 25 µg/ml), laminin (D, 20 µg/ml), Vn (E, 1 µg/ml) or fibrinogen (F, 20 µg/ml). Adhesion and spreading were monitored under phase contrast microscope. Shown are micrographs taken at 1 h post cell plating. Original magnification: x 200.*

Results

CD clone 4 cells, plated on fibronectin (25 µg/ml), vitronectin (Vn; 1 µg/ml), fibrinogen (20 µg/ml), collagen type IV (25 µg/ml), or laminin (20 µg/ml), demonstrated significant differences with respect to adhesion and spreading. On fibronectin, nearly all cells adhered within 5 min and spread within 10 min at 37°C (*Fig. 1B*). The majority of the cells (>90%) adhered onto Vn and fibrinogen within 15 min and spontaneous spreading was observed approxi-

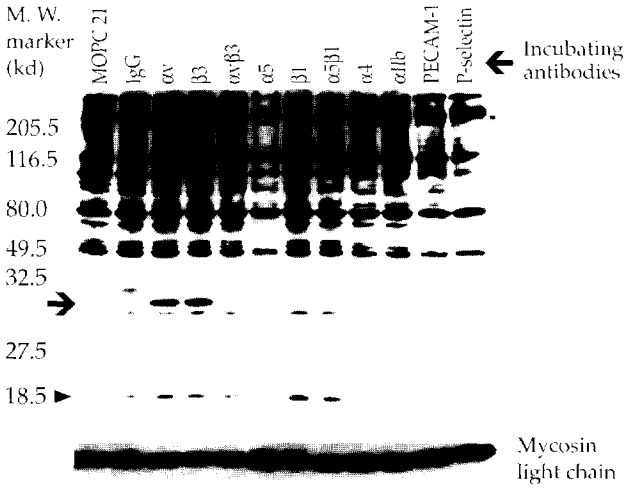


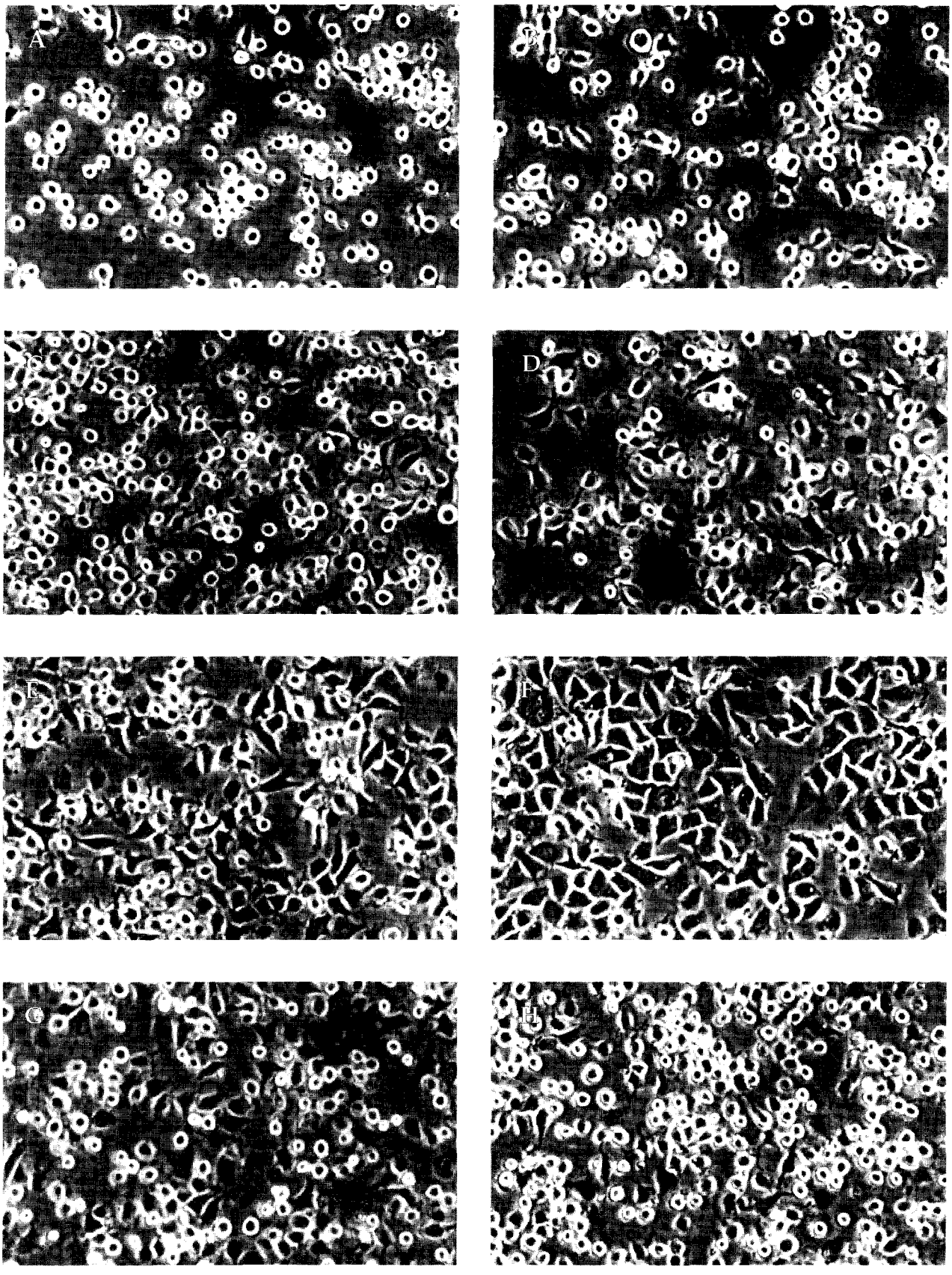
Figure 2. *CD clone 4 endothelial cells were plated on Vn (15 min; 37°C) and incubated with various mAb (5 μg/ml) or pAb (10 μg/ml) for 15 min. After extensive washing, cell lysates were prepared and used for immunoblotting with RC20H. The 30 kD protein band was indicated with an arrow and a low m.w. species (~ 20 kd) was indicated with an arrowhead. The lower panel represented the same blot stripped and reprobed with mAb to myosin light chain to demonstrate equal protein loading.*

mately 1 h after plating (*Fig.1E and F*, respectively). In contrast, CD clone 4 cells adhered poorly (<50%) to collagen IV and laminin, and demonstrated low spreading (in both the percentage of spread cells and the extent of spreading) on collagen type IV and essentially no spreading on laminin (*Fig.1C and D*, respectively). Subsequently, Vn was chosen as the adhesion substrate to explore the potential effect of ligating surface integrin receptors on cell spreading, since spontaneous spreading of CD clone 4 cells on Vn did not occur until ~1 h after plating. CD clone 4 cells were seeded onto Vn and allowed to adhere for 15 min, and then were treated with either mAbs (5 μg/ml) or pAbs (10 μg/ml) against various adhesion molecules for 15 min (*Table 1*). The specificities of these Abs have been well characterized (see *Table 1*). Ligating the cell surface αv, β3, α5, β1, or α4 integrin subunits with corresponding mAbs induced significant cell spreading, compared to control Abs MOPC or RbIgG (*Table 1*) treated cells. Rabbit pAb against α5β1 or αvβ3 induced a similar, yet less dramatic spreading (*Table 1*). The effect on enhanced spreading was specific for integrin receptors since a mAb against PECAM-1 (mAb 1.3;[22]) did not promote spreading. Similarly, mAb against P-selectin (whose expression on endothelial cell surface generally requires cell activation) and mAb to integrin αIIβ (which endothelial cells do not express) were also without effect (*Table 1*). When CD clone 4 cells were plated on collagen type IV, very similar patterns of changes in cell spreading were observed with the whole panel of Abs.

To explore the involvement of protein tyrosine phosphorylation in integrin ligation induced cell spreading, whole cell lysates were harvested for immunoblotting using anti-phosphotyrosine Abs. As presented in *Fig.2*, Abs directed to various adhesion molecules demonstrated different effects on tyrosine phosphorylation of CD clone 4 cells plated on Vn. Most significantly, incubation of CD clone 4 cells with mAb against either αv or β3 induced prominent tyrosine phosphorylation of a ~30 kD protein (*Fig.2*; arrow). All the other Abs, including mAbs against α5, α4, or β1 which also promoted cell spreading, did not induce the tyrosine phosphorylation of this protein (*Fig.3*), suggesting that β1 and β3 integrin receptors may employ different signaling mechanisms in facilitating cell spreading. Interestingly, Abs against α5, PECAM-1, and P-selectin appeared to have a slight down-regulating effect on the overall protein tyrosine phosphorylation (*Fig.2*). Less dramatically, Abs against αv, β3, β1, and α5β1 appeared to slightly increase the tyrosine phosphorylation of a ~20 kD protein, which comigrated with myosin light chain (*Fig.3*; arrowhead). When CD clone 4 cells were plated on collagen IV and treated with the above Abs, the induction of the ~30 kD tyrosine-phosphorylated protein was also observed with anti-β3 but not with anti-β1 or other antibodies (see *Fig.4 D*).

The above results suggest that ligation of β3 integrin on CD clone 4 microvascular endothelial cells induces a cell spreading process that might depend on the tyrosine phosphorylation of certain proteins such as the 30 kD protein. We thus subsequently performed a dose study of the antibody effect. Monoclonal anti-β3 Ab (OPG-2) demonstrated a dose-dependent effect in promoting CD clone 4 cell spreading on Vn (*Fig.3 A-F*). Microscopic observations revealed a dose-related transition of CD clone 4 cells plated on Vn from a rounded-up shape, to arborized, partially spread, and finally to a full spread morphology (*Fig.3 A-F*). Accompanying these morphological changes induced by mAb to β3, a dose-dependent tyrosine phosphorylation of the 30 kD protein was observed (*Fig.4 A*). The CD clone 4 cell spreading and

Figure 3. *Dose-dependent effect of β3 mAb (OPG-2) on promoting CD clone 4 cell spreading on Vn and the inhibitory effects of genistein and BAPTA. CD clone 4 cells were plated on 1 μg/ml vitronectin and allowed to adhere for 15 min before treated with increasing doses of OPG-2 (A, 0 μg/ml; B, 0.1 μg/ml; C, 0.5 μg/ml; D, 1.0 μg/ml; E, 5.0 μg/ml; F, 10.0 μg/ml) for 15 min. A, MOPC control (5 μg/ml). G. CD clone 4 cells were pretreated with 50 μg/ml genistein (10 min; 37°C) before incubated with 5.0 μg/ml OPG-2. H, CD clone 4 cells were pretreated with 10 μM of BAPTA-AM (10 min; 37°C) before incubated with 5.0 μg/ml of OPG-2. Original magnification: x 200.*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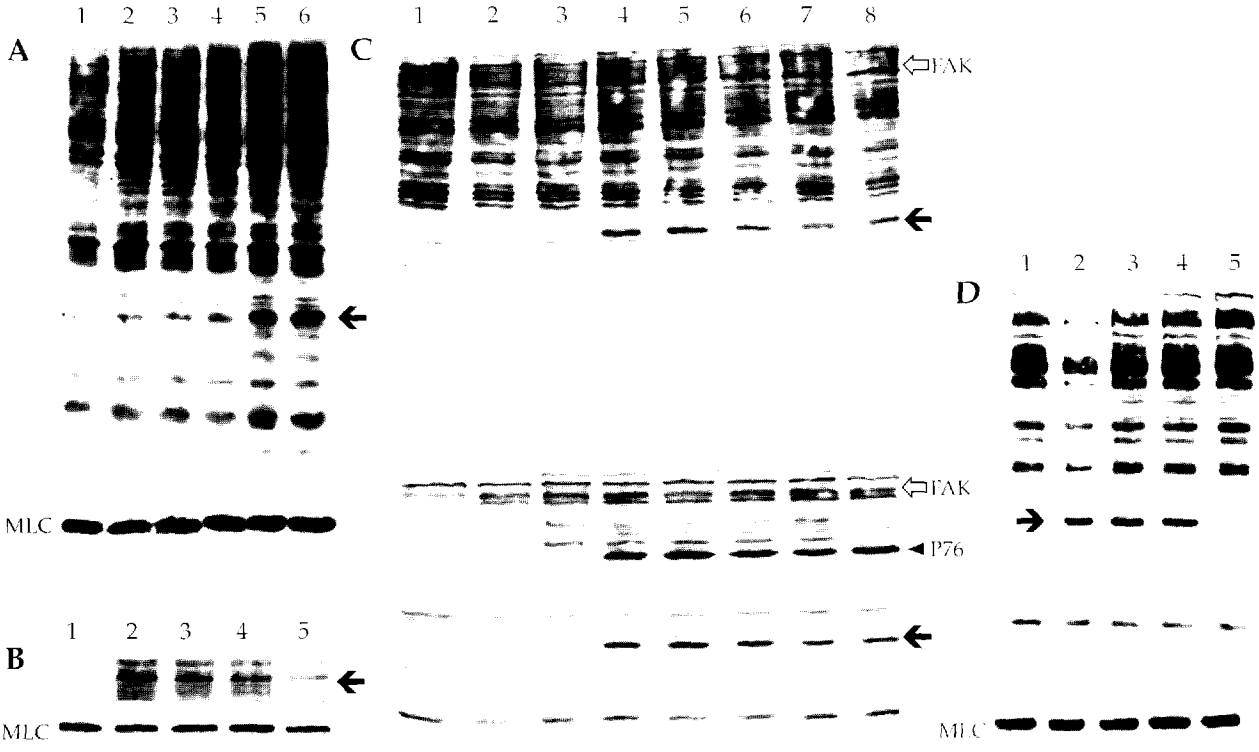

Figure 4. *A, Dose-dependent induction of the 30 kD protein (indicated by the arrow) tyrosine phosphorylation by ligating integrin β3 with OPG-2. Lanes 1-6 corresponds to samples prepared from micrographs A-F in Fig. 3. The primary antibody used was RC20H. The lower panel represents the same blot stripped and reprobed with mAb to myosin light chain to confirm equal loading of proteins. (B): Inhibition of anti-β3 Ab induced 30 kD protein (indicated by the arrow) tyrosine phosphorylation by genistein and BAPTA. CD clone 4 cells in suspension (lane 1) or adhered to Vn for 15 min (lanes 2-5) were pretreated for 10 min. with either the vehicle (DMSO) control (lane 2) or 25 μg/ml (lane 3) or 50 μg/ml (lane 4) of genistein, or 10 μM of BAPTA-AM (lane 5) followed by incubation with 5 μg/ml OPG-2 for 15 min. Cell lysates were prepared for immunoblotting with RC20H. The lower panel represents the same blot stripped and reprobed with mAb to myosin light chain. (C): Time course of the induction of the 30 kD protein tyrosine phosphorylation by OPG-2. CD clone 4 cells in suspension (lane 1) or plated on Vn (1 μg/ml) for 15 min (lanes 2-8) were incubated with either MOPC control antibody (lane 2) or with 5 μg/ml OPG-2 for 0 min (lane 3), 5 min (lane 4), 15 min (lanes 1 and 5), 30 min (lane 6), 60 min (lane 7), or 120 min (lane 8). Following extensive washing, cell lysates were prepared and used in immunoblotting with either RC20H (the upper panel) or 4G10 (the lower panel). The 30 kD protein was indicated with solid arrows, the P76 protein with an arrowhead, and FAK protein with open arrows. (D): Induction of the 30 kD protein tyrosine phosphorylation by OPG-2 in CD clone 4 cells plated on collagen IV. CD clone 4 cells adhered to collagen IV (25 μg/ml) for 30 min were incubated with MOPC ascites (15 min; lane 1), 5 μg/ml OPG-2 for 5 (lane 2), 15 (lane 3) or 60 min (lane 4), or mAb to β1 (15 min; lane 5). Cell lysates were prepared and used in immunoblotting with RC20H. The 30 kD protein was indicated with an arrow. The lower panel represents the same blot stripped and reprobed with mAb to myosin light chain.*

tyrosine phosphorylation of the 30 kD protein induced by ligating β3 integrin were not further enhanced by clustering (or crosslinking) the receptors[19] with a secondary Ab (data not shown). Pretreatment of CD clone 4 cells with genistein, a general tyrosine kinase inhibitor, partially inhibited OPG-2 induced cell spreading (*Fig. 3 G*) and the 30 kD protein tyrosine phosphorylation (*Fig.4 B*, lanes *3* and *4*). In contrast, pretreatment with BAPTA-AM, a cell membrane-permeable intracellular Ca^{2+} chelator, completely inhibited the OPG-2 effects (*Fig.3 H* and *Fig. 4 B*). A time study using RC20H anti-phosphotyrosine Ab demonstrated that the tyrosine phosphorylation of the 30 kD protein occured and peaked within 5 min after Ab ligation and remained prominent by 2 h (*Fig.4 C*, upper panel). Interestingly, immunoblotting with another anti-phosphotyrosine Ab 4G10 detected, in addition to the 30 kD protein, another 76 kD tyrosine-phosphorylated protein after anti-β3 Ab treatment (*Fig.4 C*, the lower panel). Both RC20H and 4G10 detected a ~120 kD protein whose tyrosine phosphorylation did not change upon integrin ligation (*Fig.4 C*; indicated by open arrows). This protein most likely represents the FAK (focal adhesion kinase; $pp125^{FAK}$),[18,19] since its tyrosine phosphorylation was unique to adherent cells and reprobing the same two blots in *Fig.4 C* with a mAb to FAK (i.e., 2A7) revealed a single protein band at the same position across all the lanes. Incubation of both RC20H and 4G10 with 5 mM phosphotyrosine, but not with phosphoserine or phosphothreonine,

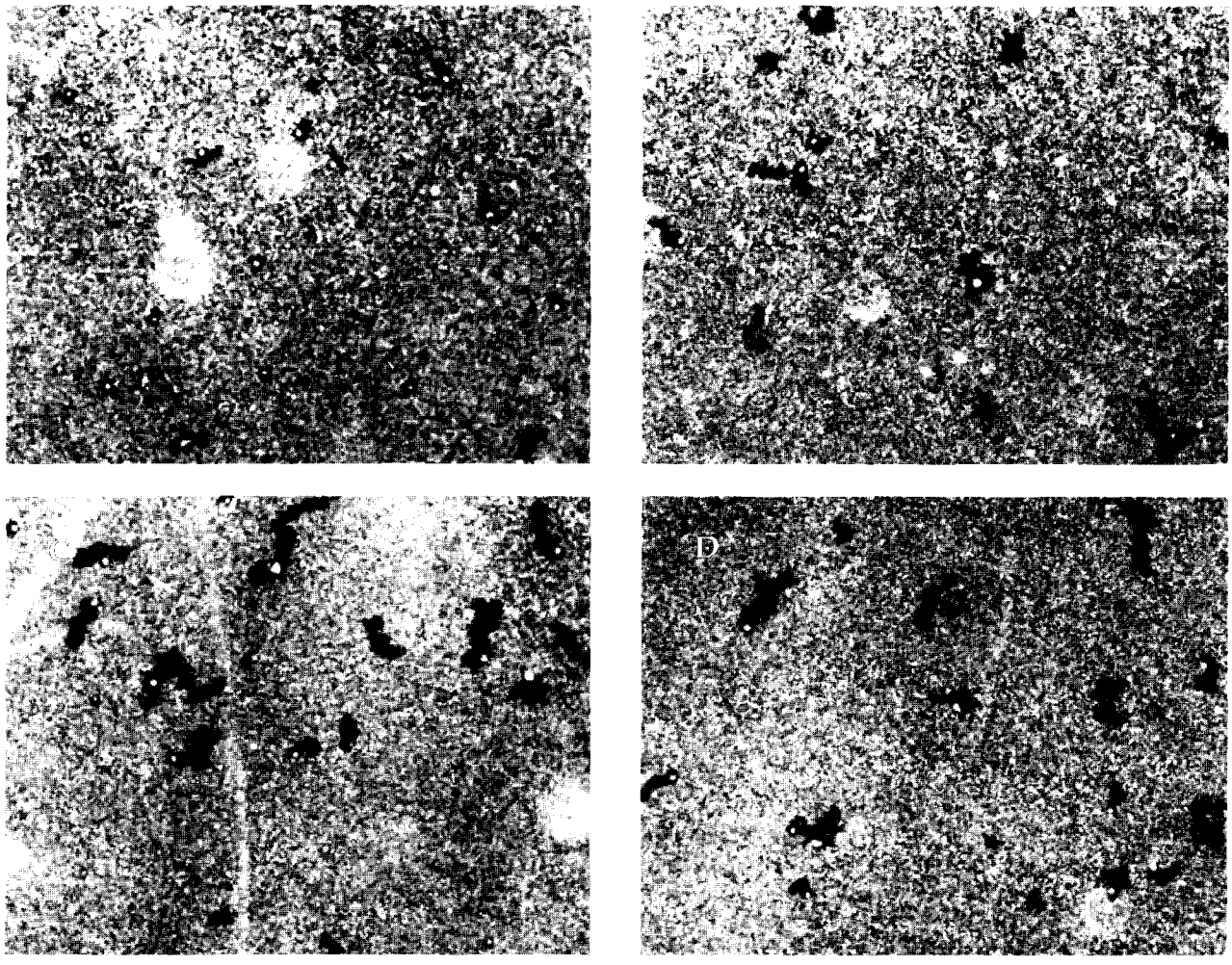

Figure 5. *Integrin ligation induced CD clone 4 cell motility on collagen IV. CD clone 4 cells were plated for 1 h on collagen IV on which a thin carpet of gold particles was plated.[23] Then cells were treated with 5 μg/ml of MOPC (A), or mAbs to αv (B), β3 (C) or β1 (D) for 15 min. Thereafter cells were cultured in serum-free DMEM for up to 48 h. Random cell motility was assessed by the area cells had cleared.[23] Shown were micrographs of cells 48 h post antibody treatment. Original magnification: x100.*

abolished the reactivity of these antibodies, thus confirming their specificty towards phosphotyrosine.

The β3 integrin ligation induced tyrosine phosphorylation of the 30 kD protein and cell spreading did not appear to depend on the substrate onto which CD clone 4 cells were initially plated. Cells plated on collagen IV also demonstrated the induction of the 30 kD protein tyrosine phosphorylation (*Fig.4 D*) and an enhanced spreading (*Table 1*) in response to receptor ligation with OPG-2, which also had a similar effect on CD clone 4 cell spreading on intact SEM (data not shown). Cell spreading is accompanied by the establishment and organization of cytoskeleton and generally is considered a preparatory and prerequisite step for cell motility/migration. Thus, we subsequently examined the effect of integrin conjugation on CD clone 4 motility using the phagokinetic track motility assay.[23] Ligating either β1 or β3 integrins induced random cell motility on both collagen IV (*Fig.5*) and Vn (*Table 1*). Polyclonal Abs to αvβ3 and α5β1, and a mAb to α4 also demonstrated moderate motility-enhancing efficacy while all other Abs tested were without effect (*Table 1*).

Discussion

The major findings of this study were two fold. First, ligation of both β1 and β3 integrin receptors induced significant endothelial cell spreading and motility in a substrate independent manner. Second, only ligation of β3 (but not β1) integrins induced a prominent tyrosine phosphorylation of a 30 kD protein which temporally preceded CD clone 4 cell spreading and motility, suggesting that these two integrin receptors may utilize distinct intracellular signaling mechanisms in modulating endothelial cell function such as spreading and motility. The current study provides a possible experimental explanation for previous observations that β1 and β3 integrins play distinct roles in the

adhesion/spreading/motility of vascular cells such as smooth muscle cells and endothelial cells[13-27] The spreading/motility-enhancing effect appears to be specific for vascular integrins (*Table 1*) since control Ab MOPC. Ab against integrin molecule that endothelial cells normally do not express (i.e., αIIβ), Ab against an adhesion molecule whose expression on endothelial cell surface requires prior cellular stimulation (i.e., P-selectin), and Ab against an adhesion molecule that CD clone 4 cells normally express on the surface (i.e., PECAM-1) all had no effect. The induction of the tyrosine phosphorylation of the 30 kD protein is unique to the conjugation of integrin αvβ3 since all other antibodies, including Abs against α4, α5, and β1 which bound to CD clone 4 cells[22] and induced cell spreading/motility (*Table 1*), did not effect the induction. This specificity also excluded the possible involvement of Fc receptors. Interestingly, a rabbit polyclonal anti-αvβ3 Ab did not induce the 30 kD protein tyrosine phosphorylation (see *Fig.3*) although it induced cell spreading/motility. The reason for this paradox ou remains to be determined. However, it is quite possible that the tyrosine phosphorylation of the 30 kD protein induced by conjugating the surface receptor (by the Ab) is epitope- and/or conformation-dependent, i.e., mAb to αv or β3 (but not pAb to αvβ3) binds to a unique epitope on αvβ3 such that, with or without conformational changes, the receptor is "activated" to initiate the signal transduction (i.e., the tyrosine phosphorylation of the 30 kD protein). The enhanced cell spreading/motility and induction of the 30 kD protein tyrosine phosphorylation appear to require only bivalent ligation of the integrin receptor but not crosslinking via a secondary Ab. This type of receptor activation has previously been observed in a variety of experimental systems.[21,25]

Tyrosine phosphorylation of the 30 kD protein temporally precedes endothelial cell spreading, thus suggesting a potential cause-and-effect relationship between the two events. Both the 30 kD protein tyrosine phosphorylation and Ab-induced CD clone 4 cell spreading/motility could be inhibited, in a dose-dependent fashion, by tyrosine kinase inhibitor, genistein, therefore strengthening the notion that CD clone 4 spreading induced by conjugation of integrin αvβ3 is a tyrosine-phosphorylation dependent process. Also, both events (i.e., tyrosine phosphorylation of the 30 kD protein and cell spreading) could be completely blocked by removal of intracellular calcium suggesting that the αvβ3-transmitted signals rely on calcium transients thus confirming previous reports on the role of this integrin receptor in mobilizing intracellular calcium.[12,28] The identity of the 30 kD protein (and some other induced proteins such as the 76 kD protein) remains to be established. Suffice it to say that the tyrosine phosphorylation of the 30 kD protein (and p76) appears to be specifically involved in β3 integrin signaling pathway and only occurs, like $pp125^{FAK}$, in adherent cells (see *Fig.4 B and C*). However, unlike $pp125^{FAK}$ which undergoes tyrosine phosphorylation immediately upon cell adhesion to the substrate, the tyrosine phosphorylation of the 30 kD protein is observed only after further ligation of surface β3 integrins in adherent cells. It will be of interest to determine whether the 30 kD protein is one of the substrates of $pp125^{FAK}$.

One may wonder why CD clone 4 endothelial cells plated on Vn (via αvβ3 and/or αvβ5 binding) still respond to receptor ligation by anti-αv or anti-β3 Abs. Two non-exclusive possibilities may provide the explanation. First, unlike most other studies where very high concentrations of Vn (10–40 μg/ml) were employed,[12,13] we used 1 μg/ml of Vn substrate which would not saturate all αvβ3 receptors and thus leave a population of αvβ3 receptors on the membrane. Second, adhesion of CD clone 4 cells to matrix proteins would trigger intracellular mechanisms which provide a positive feedback[26] with respect to the adhesive potential. This may upregulate the cell surface expression of integrin αvβ3. The fact that the Ab-ligation induced protein tyrosine phosphorylation and cell spreading/motility was to a degree substrate-independent provides support to the second possibility. The current study suggests a possible in vivo scenario wher endothelial cell phenotypes such as spreading and motility, which are important parameters in tumor angiogenesis and other diseases such as atherosclerosis, could be modulated when endothelial cells interact with other cell types (tumor cells, smooth muscle cells, platelets, etc) using integrin receptors.

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