VLA-4 Integrin Cross-linking on Human Monocytic THP-1 Cells Induces Tissue Factor Expression by a Mechanism Involving Mitogen-activated Protein Kinase*

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Adhesion molecules such as VLA-4 are important not only for monocyte adhesion to extracellular matrix proteins, but also for subsequent cell activation. Monocyte adherence to fibronectin or engagement of VLA-4 has been demonstrated to stimulate production of potent inflammatory mediators such as tumor necrosis factor-α, interleukin-1, and the procoagulant tissue factor protein. However, the intracellular signaling cascades leading to gene expression have not been elucidated. Using the human monocytic THP-1 cell line, VLA-4 cross-linking by monoclonal antibodies directed against its α4 and β1 subunits produced a time-dependent increase in tyrosine phosphorylation of a broad range of cellular proteins. Using Western blot analysis directed against the phosphorylated form of the extracellular signal-related kinase (ERK) mitogen-activated protein (MAP) kinase proteins, as well as immunoprecipitation and in vitro kinase assays, we found that VLA-4 cross-linking increased ERK1/ERK2 tyrosine phosphorylation and activity. In conjunction, integrin cross-linking also increased NF-κB nuclear translocation and 4-h expression of tissue factor. Inhibition of tyrosine kinase activity with genistein (10 μg/ml) as well as selective MAP kinase inhibition with the MEK-1 inhibitor PD98059 abolished the VLA-4-dependent ERK tyrosine phosphorylation, inhibited NF-κB nuclear binding, and abrogated tissue factor expression induced by both VLA-4 cross-linking and adhesion to fibronectin in THP-1 cells and human peripheral blood monocytes. These studies point to the involvement of the MAP kinase pathway in the activation of monocytic cells during transmigration to inflammatory sites.

The integrin family of surface adhesion molecules plays a key role in leukocyte recruitment to areas of extravascular inflammation. These heterodimeric integral membrane proteins are important not only for the adhesion to and transmigration across endothelial barriers, but also for adhesive interactions with extracellular matrix proteins (1–3). While initially considered important only for their adhesive properties, recent studies have suggested that integrin engagement can initiate signal transduction pathways contributing to cellular activation (4–8).

Monocyte recruitment to extravascular sites is an important component of the host response to a variety of stimuli including bacterial infection, tumor deposits, and atherosclerotic plaques. In this location, the surface expression as well as release of a number of macrophage products serve to coordinate the local inflammatory response. Fibrin deposition induced by macrophage tissue factor (TF) expression is known to contribute significantly to the development of this response. Within the vascular space, adherence of monocytes to the endothelium stimulates expression of TF on monocytes, a process that likely contributes to local microvascular thrombosis (9, 10). Furthermore, at extravascular sites, products of both the coagulation and fibrinolytic cascades contribute to the generation of the inflammatory response through their interaction with infiltrating cells. Indeed, strategies aimed at reducing fibrin deposition or precluding TF expression have been shown to mitigate the full expression of both the local and systemic inflammatory response depending on the model system studied (9, 11–15).

Recent work has defined a role for integrin engagement in the induction of monocyte TF expression, as well as other immediate early genes such as interleukin-1β (16), interleukin-8, and tumor necrosis factor, and (8) transcription factors IκB (11), c-Jun, and c-Fos (17). While monocytes are endowed with a variety of surface integrins, engagement of very late antigen 4 (VLA-4) appears to consistently induce gene expression (16–23). For example, ligation of VLA-4 by monoclonal antibody in both human peripheral blood monocytes (PBM) and in the monocytic THP-1 cell line promotes TF expression, whereas engagement of β2 integrins has little effect (20). The intracellular signaling mechanisms leading to the VLA-4-mediated induction of TF as well as other inflammatory genes appears to involve the induction of tyrosine phosphorylation (16, 18, 24). Recent studies by Lin and colleagues have implicated a possible signaling role for Syk tyrosine kinase in this process (18). In human monocytic THP-1 cells, VLA-4 engagement caused prominent tyrosine phosphorylation as well as activation of Syk tyrosine kinase, an effect that occurred in concert with the induction of the interleukin-1β gene.

The abbreviations used are: TF, tissue factor; ERK, extracellular signal-related kinase; MAP, mitogen-activated protein; PBM, peripheral blood monocytes; MEK, MAP kinase kinase; LPS, lipopolysaccharide; FCS, fetal calf serum; mAb, monoclonal antibody; Ab, antibody; PCA, procoagulant activity; PMSF, phenylmethylsulfonyl fluoride; MBP, myelin basic protein; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis.

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Integrin engagement through interaction with extracellular matrix proteins has been shown to contribute to the regulation of cellular growth and differentiation and to modulate tumor behavior (1). In fibroblasts, this process involves tyrosine phosphorylation and activation of MAP kinase through a cascade involving Raf-1 and MEK (1, 25–27). While this cascade is known to be activated in macrophages in response to various proinflammatory stimuli (28–35), its contribution to the β2 integrin-induced activation of inflammatory cell gene expression is unknown. In the present studies, engagement of VLA-4 on the surface of THP-1 monocytic cells and on human monocytes, both through integrin cross-linking and attachment to a fibronectin substratum, was shown to induce tyrosine phosphorylation and activation of the ERK1/ERK2 MAP kinases. This effect occurred in parallel with nuclear translocation of NF-κB and stimulation of TF on the surface of these cells. Furthermore, we took advantage of the existence of a novel selective inhibitor of MEK1 to define the contribution of this pathway to integrin-induced TF expression. This agent, PD98059, caused a dose-dependent inhibition of tyrosine phosphorylation and activation of MAP kinase in response to VLA-4 engagement and concomitantly prevented NF-κB translocation and TF induction. Considered together, these studies support a contribution of the MAP kinase pathway to integrin-induced gene expression in cells of monocyte/macrophage lineage.

**EXPERIMENTAL PROCEDURES**

**Buffers and Reagents**

Genistein was purchased from Calbiochem and prepared in Me₂SO at 10 mg/ml. The selective MEK1 inhibitor PD98059 was the kind gift of Dr. R. Saltiel, and was prepared in Me₂SO. Escherichia coli O111:B4 lipopolysaccharide (LPS) was purchased from Life Technologies, Inc., as were endotoxin-free RPMI and HBBS media. Fetal calf serum (FCS) was from HyClone. The following antibodies were used in the integrin engagement studies: mouse IgG1 anti-CD49d (mAb HP2.1 (Immunotech) and mAb 44H6 (Serolect)), CD29 (mAb R20 and MAb L1a.2 (Immunotech)), goat Polyclonal anti-IgG (Immunotech), mouse IgG1 anti-CD45 mAb 1214 (PDI Bioscience), and negative mouse IgG1 (Sigma). Inhibitory anti-tissue factor antibody (mAb 4909) was obtained from American Diagnostica.

**Cell Preparation**

Human monocyte THP-1 cells (ATCC) were propagated in RPMI/10% FCS/penicillin/streptomycin at 37 °C, 5% CO₂. Human PBMC were isolated from the blood of normal healthy donors by centrifugation over a Ficoll-Hypaque gradient at 400 x g for 20 min. The mononuclear layer was aspirated, washed twice and resuspended in RPMI/2% FCS/0.1% glutamine. This cell population contained 25–35% monocytes as assessed by Wright’s stain and CD14 expression (flow cytometry with fluorescein isothiocyanate-conjugated anti-CD14 Ab; Becton-Dickinson), with >96% viability by trypan blue exclusion and propidium iodide uptake.

**Cell Activation**

For integrin engagement studies, THP-1 cells were suspended in RPMI/2% FCS/L-Glu at 5 x 10⁶ cells/ml. Surface CD29, CD49d, and CD45 antigens were ligated with monoclonal antibody for 25 min at 15 °C. Poly-L-lysine was cross-linked under UV light for 2 h; all wells were stopped with the addition of 2 x 10⁶ cells/ml RPMI/2% FCS/0.1% glutamine for 45 min at 4 °C. Protein G-Sepharose was added and incubated at 4 °C for 1 h. The supernatants were collected following centrifugation at 10,000 x g for 5 min and diluted with 2 x 10⁶ cells/ml RNAse buffer, 0.1% diethylthreitol (DTT). Following adhesion to poly-L-lysine or fibronectin substrata, PBMC were lysed in the culture wells with ice-cold lysis buffer and prepared in a similar fashion. Lysates prepared from 100,000 cells were separated on 12.5% SDS-PAGE and transferred to polyvinylidene difluoride membrane (Mobilion). Blots were then probed with polyclonal rabbit (Transduction Laboratories) anti-phosphotyrosine antibody, rabbit anti-phospho-ERK antibody (Santa Cruz Biotechnology). Following incubation with the appropriate horseradish peroxidase-conjugated secondary antibody (Sigma), blots were developed using an ECL-based system (Amersham).

**Phosphotyrosine and ERK Immunoprecipitations—**

**Immunoprecipitations and Immune Complex Kinase Assays**

Phosphotyrosine and ERK Immunoprecipitations—Cells (3 x 10⁶) were seeded above the porous table supernatant preincubated with Protein G-Sepharose (Pharmacia Biotech Inc.). Cellular proteins were immunocomplexed using polyclonal anti-phosphotyrosine antibody (Transduction) or anti-ERK-1 and -ERK-2 antibody (SCB) for 1 h at 4 °C. Protein G-Sepharose was added and incubated at 4 °C for 1 h. The resulting immune complexes were washed five times with cold phosphate-buffered saline/0.1% Tween 20, and then separated from beads by 2 x Laemmli buffer, 0.1% DTT, and boiling at 100 °C for 5 min. Beads were then sedimented by ultracentrifugation and the supernatant collected for Western blot analysis.

**ERK-2 Immune Complex Kinase Assays—**

ERK-2 immunocomplexes were washed with five changes of cold phosphate-buffered saline/0.1% Tween 20, and then incubated for 30 min at 30 °C with 20 μg of anti-ERK-2 monoclonal antibody (MPB, Upstate Biotechnology, Inc.) in kinase assay buffer composed of 0.4 mM cold and 0.4 μM [γ-32P]ATP (DuPont NEN), 50 mM Tris-HCl (pH 7.4), and 10 μM MgCl₂. Reactions were stopped with the addition of 2 x Laemmli buffer, 0.1% DTT and boiling at 100 °C for 5 min. Equal volumes were loaded and run on 10% SDS-PAGE. The radioactivity of the phosphorylated MPB band running at 20 kDa was quantified on a Molecular Dynamics SI PhosphorImager.

**Preparation of Nuclear Extracts**

Following cell activation by VLA-4 cross-linking, 5 million THP-1 cells were washed twice in cold HBSS and lysed in 10 μM HEPES (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM DTT, 0.5 mM PMSF, and 0.1% Nonidet P-40. Following centrifugation at 13,000 rpm (4 °C) for 10 min, the nuclear pellet was resuspended in 15 mM HEPES (pH 7.9), 25% glycerol, 420 μM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, 0.5 mM spermidine, 0.15 mM spermine, and 5 μg/ml each of leupeptin, pepstatin, and aprotinin. Supernatants were collected after a 15-min centrifugation at 14,000 rpm (4 °C) and diluted with 75 μl of buffer containing 20 mM HEPES (pH 7.9); 20% glycerol, 0.2 mM EDTA, 50 mM KCl, 0.5 mM DTT, and 0.5 mM PMSF and immediately frozen on dry ice. Protein concentrations were determined using the Bradford protein assay (Bio-Rad).

**Electrophoretic Mobility Shift Assay**

5 μg of nuclear extract protein were preincubated with the nonspecific DNA competitor poly(dI-dC) (5 μg, Pharmacia) for 10 min at room temperature. [γ-32P]Radiolabeled probe containing 2 NF-κB sites derived from the human immunodeficiency virus-1 enhancer (HIV-ENH), or containing the TP-specific NF-κB site (49), was incubated for an addi-
FIG. 1. VLA-4 ligation and cross-linking induces tyrosine phosphorylation. A, time course. THP-1 surface VLA-4 was cross-linked using either mAb HP2/1 or K20 and the anti-mouse F(ab′)2 fragment. The cells were incubated for the times indicated, lysed, and the lysates probed for phosphotyrosine residues by Western blot. B, cross-linking versus ligation. VLA-4 was engaged by various mAb either by cross-linking or simple ligation, and cell lysates probed for phosphotyrosine residues. C, controls. The phosphotyrosine accumulation induced by cross-linking the β1 (K20) or α4 (HP2/1) VLA-4 subunits was compared with that induced by cross-linking CD45 (mAb 1214), or by incubating cells in the presence of goat anti-mouse F(ab′)2 or negative mouse IgG. Cells were lysed at 5 min in each case. D, epitope-specific PTK activation. VLA-4 was engaged by cross-linking β1 (K20, Lial1.2) and α4 (HP2/1, 44H6) subunits. THP-1 cells were lysed 5 min after cross-linking and probed for phosphotyrosine accumulation.

Induction of Tyrosine Phosphorylation by Cross-linking VLA-4—Fig. 1A demonstrates that cross-linking of either the β1 or α4 subunits of VLA-4 induces a time-dependent increase in phosphotyrosine accumulation in THP-1 cells. The increase occurred within 1 min, reached a maximum at 5–10 min, and persisted for ~30 min. While receptor ligation alone caused a small increase in phosphotyrosine residues, cross-linking markedly amplified this effect (Fig. 1B). This effect was not due to the addition of the secondary antibody per se, since this did not induce phosphotyrosine accumulation (Fig. 1C). We also compared the effect of cross-linking using antibodies directed against different epitopes of the α4β1 integrin. As shown in Fig. 1D, cross-linking of α4β1 with the inhibitory antibody Lial1.2 directed against the β1 subunit caused a similar pattern of phosphotyrosine accumulation compared with the non-inhibitory antibody K20, although the magnitude was somewhat less. HP2/1, an inhibitory antibody directed against the α4 subunit caused similar accumulation of phosphotyrosine residues, while use of 44H6, also directed against α4, failed to induce tyrosine phosphorylation despite surface binding equivalent to mAb HP2/1. (data not shown, as assessed by flow cytometry, Coulter Epics MCL). Finally, the effect was not due to the process of cross-linking of surface antigen per se, since neither cross-linking with mAb 1214 (Fig. 1C) nor 44H6 induced an increase in tyrosine-phosphorylated proteins (Fig. 1D). As for 44H6, mAb 1214 had surface binding characteristics equivalent to K20 and HP2/1 (data not shown, as assessed by flow cytometry).

Endotoxin is known to cause tyrosine phosphorylation in monocyteic cells (31, 34). The observed effect of cross-linking was unlikely to be due to endotoxin contamination of the system. Polymyxin B did not reverse the stimulation of phosphotyrosine accumulation, and heating of the primary antibody prior to incubation with the cells completely prevented induction of tyrosine phosphorylation. Furthermore, as indicated above, neither primary nor secondary antibody alone mimicked the stimulatory effect.

Tyrosine Phosphorylation and Activation of ERK1/ERK2 following VLA-4 Cross-linking—While cross-linking induced a pattern of tyrosine phosphorylation encompassing a broad range of proteins, cross-linking with K20 caused some degree of persistent phosphorylation in the 42–50-kDa range. To determine whether p44 ERK1/p42 ERK2 MAP kinases might be among the candidate substrate proteins, cell lysates were probed first for phosphotyrosine residues, and the blots stripped and reprobed for p44 ERK2 and p44 ERK1. Both ERK proteins comigrated with an area of VLA-4-induced persistent tyrosine phosphorylation (data not shown). Importantly, the absolute amount of ERK protein in the cell lysates did not change over time following integrin engagement.

To conclusively identify the ERK proteins as targets of the VLA-4-induced tyrosine phosphorylation, two approaches were used. First, immunoprecipitation studies were carried out at 5 min after cell stimulation. The upper panel of Fig. 2A shows a blot of immunoprecipitated tyrosine phosphoproteins probed with anti-ERK2 Ab under each of the treatment conditions. Integrin cross-linking with K20 markedly enhances the amount of tyrosine-phosphorylated ERK2 protein compared with control cells, while engagement alone causes a slight increase. A similar effect is observed when cell lysates are sedimented with either anti-ERK1 Ab or anti-ERK2 Ab and then probed with anti-phosphotyrosine Ab (Fig. 2A, middle and lower panels, respectively). Similar results were obtained when
cell surface integrins were cross-linked with mAb against the α subunit (data not shown). ERK phosphorylation induced by VLA-4 cross-linking was also evaluated by Western blot studies using an antibody specific to ERK phosphorylated on tyrosine residues. Cross-linking of VLA-4 by mAb against the α subunit induced a time-dependent increase in ERK tyrosine phosphorylation, particularly of the p42 ERK2 (Fig. 2B). This effect occurred early, peaked at 30 min, and persisted for at least 60 min. This was accompanied by a slight retardation in the electrophoretic mobility, consistent with phosphorylation of ERK2. Integrin ligation alone caused a somewhat delayed rise in phospho-ERK accumulation, although cross-linking induced more phosphorylation at any given time point.

Having demonstrated tyrosine phosphorylation of ERK1/ERK2 proteins, studies were performed to evaluate their level of activation. Using MBP as a substrate, Fig. 3 (A and B) shows that cross-linking of α and β subunits of VLA-4, respectively, cause a time-dependent increase in immunoprecipitated ERK2 activity, the magnitude of which is similar to that seen with 1 μg/ml LPS. Similar results were seen with immunoprecipitated ERK-1, although the increase in activity stimulated by VLA-4 engagement was less, approximately 2–3-fold (data not shown). As expected from the pattern of induction of phosphorytrosine accumulation, cross-linking mAb L1a.2 markedly increased immunoprecipitated ERK2 activity, while cross-linking 44H6 or mAb 1214 had little to no effect (data not shown).

**FIG. 2. VLA-4-induced ERK tyrosine phosphorylation.** A, THP-1 cells were lysed 5 min after engagement of VLA-4 by mAb K20, following which ERK1, ERK2, or proteins phosphorylated on tyrosine residues were immunoprecipitated. Western blotting was performed as indicated. Similar results were obtained with mAb HP2/1. B, following VLA-4 engagement THP-1 cells were lysed at the times indicated and prepared for Western blotting with Ab specific to phosphorylated ERK. Note that while both ligation (binding) and VLA-4 cross-linking induce the same pattern of ERK phosphorylation, cross-linking induces considerably more at any given time. All studies were replicated a minimum of three times.

**FIG. 3. Activation of ERK2 by VLA-4 engagement.** The VLA-4 integrin on THP-1 cells was engaged by cross-linking the α4 (mAb HP2.1) or β2 (mAb K20) subunit, and the cells incubated at 37 °C for the times indicated. For comparison, cells were treated with 1 μg/ml LPS for 30 min. Following cell lysis, ERK-2 protein was immunoprecipitated as described under “Experimental Procedures,” and the immunocomplexes allowed to phosphorylate ultra-pure MBP for 30 min at 30 °C. Proteins were then separated on 15% SDS-PAGE. A and B, time course of ERK-2 activation by VLA-4 engagement: the upper panels are autoradiograms of the 20-kDa [32P]MBP band, and the lower panels represent the radioactivity of the corresponding bands as determined by phosphomager. The data shown are representative of two independent experiments for each antibody.

**FIG. 4. Induction of PTK activity and ERK phosphorylation by matrix adhesion.** A, human PBM were allowed to adhere either to poly-L-lysine (PL) or fibronectin (FN). Cell lysates taken at the times indicated were probed with anti-phosphotyrosine Ab. B, human PBM were allowed to adhere to fibronectin or poly-L-lysine for the times indicated, when they were lysed and ERK-2 was immunoprecipitated. The resulting blot was probed with anti-phosphotyrosine Ab. In parallel experiments it was determined that over the first 30 min, >70% of all the cells of the human PBM/lymphocyte layer that bound to the fibronectin or poly-L-lysine matrices were monocytes by CD14 analysis on the flow cytometer.
VLA-4 Induces Monocytic Tissue Factor via MAP Kinase

**FIG. 5.** Inhibition of MAP kinase activity and tyrosine phosphorylation by PD98059. A, following preincubation with PD98059 or genistein, THP-1 cells were lysed 30 min after K20 engagement of VLA-4, and ERK2 was immunoprecipitated. Immunocomplexes were assayed for MBP phosphorylation activity as described; [32P]MBP autoradiograms are presented. B, immunoprecipitated p42 ERK2 activity 30 min after VLA-4 engagement by mAb HP2/1. C, THP-1 cells were preincubated with PD98059, and VLA-4 engaged by K20 or HP2/1 cross-linking. Cells were lysed 20 min after cross-linking, and lysates probed for ERK phosphorylated on tyrosine residues. D, THP-1 cells were preincubated with genistein or PD98059, and VLA-4 engaged by K20 cross-linking. Cells were lysed 5 min after cross-linking and lysates probed for phosphotyrosine residues.

**FIG. 6.** Tissue factor induction by integrin engagement and matrix adhesion. A, the VLA-4 integrin on THP-1 cells was engaged either with mAb alone (ligation), or with mAb cross-linked with Fab' secondary Ab, and cells were harvested after 4 h for PCA determination. B, THP-1 cells or human PBM were allowed to adhere to poly-L-lysine or fibronectin matrices for 4 h, and then harvested for PCA assessment. The cumulative results of three separate experiments are shown; data: mean ± S.E., n = 6–8/group. ***, p < 0.001 versus control (ANOVA with Tukey); **, p < 0.001 versus control (Student’s T-test).
2/1 cross-linking and 1 m expression of the procoagulant molecule tissue factor contributes post hoc was performed by one-way ANOVA with and are presented as mean S.E., n = 6–8/group. Statistical analysis was performed by one-way ANOVA with post hoc Tukey.

did not reverse the effect of cross-linking.

Fig. 7 shows the effect of PD98059 and genistein on VLA-4-induced PCA. At concentrations of PD98059 that preclude ERK activation in response to integrin cross-linking (10 μM for HP 2/1 cross-linking and 1 μM for K20 cross-linking), this inhibitor prevented induction of PCA (Fig. 7A). Similarly, genistein prevented the rise in PCA following cross-linking of VLA-4 (Fig. 7A), at a concentration that caused near complete inhibition of MBP phosphorylation. Fig. 7B demonstrates the ability of PD98059 to abrogate the rise in PCA that occurs in response to attachment to a fibronectin substrate in both THP-1 cells and human monocytes.

**VLA-4-induced NF-κB Nuclear Translocation Is Dependent on ERK Activation—**Previous studies have reported that VLA-4 engagement dramatically increases NF-κB nuclear translocation and specific binding in THP-1 cells (20). Since NF-κB binding to the promoter of the tissue factor gene is required for induction of gene transcription, we examined whether PD98059 might exert its effect through inhibition of NF-κB translocation. Fig. 8 illustrates two representative studies. Cross-linking VLA-4 induces a dramatic increase in both HIV-ENH and TF-specific NF-κB translocation. Preincubation of the cells with 10 μM PD98059 largely abolished the HIV-ENH shift, and consistently effected a partial inhibition of the TF κB shift.

**DISCUSSION**

Macrophage-mediated fibrin deposition via the surface expression of the procoagulant molecule tissue factor contributes significantly to the pathogenesis of both intravascular and extravascular inflammation. Induction of tissue factor occurs in response to a variety of proinflammatory stimuli including tumor necrosis factor, C3a, formyl peptides, as well as various bacterial species and their surface components (9, 11). Recent studies including those reported here demonstrate that engagement of monocyte surface VLA-4 by specific antibody is able to induce tissue factor expression (20). The present results clearly demonstrate that tyrosine phosphorylation and activation of ERK1/ERK2 MAP kinase are involved in integrin-induced signaling pathway leading to tissue factor expression. Several lines of evidence support this conclusion. First, integrin aggregation through cross-linking causes tyrosine phosphorylation of these proteins. This was definitively shown by immunoprecipitation studies, as well as by experiments using an antibody directed against the phosphorylated form of ERK. Furthermore, adhesion of both THP-1 and human monocytes to fibronectin caused phosphorylation of ERK. Second, VLA-4 cross-linking caused a time-dependent activation of ERK1/ERK2 MAP kinase as assessed by its ability to phosphorylate its substrate protein myelin basic protein. Finally, two strategies shown to prevent phosphorylation and activation of ERK precluded the induction of tissue factor, not only in response to cross-linking, but also following adhesion to fibronectin. These included the use of the tyrosine kinase inhibitor genistein as well as the novel specific inhibitor of the upstream activator of ERK, PD98059. Considered together, these findings invoke a role for the MEK-1/MAP kinase cascade in the integrin-induced activation of monocyte coagulation molecules.

The data presented here are consistent with those recently reported by Lin and colleagues showing that adhesion of THP-1 monocytes to fibronectin lead to the tyrosine phosphorylation of pp125FAK, paxillin, and the nonreceptor tyrosine kinase Syk (18, 39). Syk phosphorylation was also associated with its activation. At least two pathways leading to the activation of the Ras signaling cascade may link these effects to the activation of MAP kinase demonstrated in this study. First, phosphorylation of FAK creates an SH2-binding site for Grb2, resulting in localization of Grb2-SOS complexes and subsequent activation of Ras (40). In addition, in cultured mast cells, activated Syk has been shown to cause tyrosine phosphorylation of Shc in response to engagement of the Fc εRI with antigen (41). Consequent association with Grb2 may lead to Ras activation. The
precise signaling pathways leading to MAP kinase activation following integrin engagement, however, require further definition. While the activation of MEK-1/MAP kinase following integrin engagement in mononuclear cells suggests the involvement of the Ras as well as the downstream kinase Raf in the signaling pathway, two lines of evidence suggest that the pathway does not align precisely along the lines suggested for growth factor-induced cell signaling. For example, a recent study using NIH 3T3 fibroblasts demonstrated activation of Raf-1, MEK-1, and MAP kinase following adhesion to fibronectin in a manner that was independent of Ras (42). Second, the studies reported by Lin and colleagues demonstrated that neither phosphorylation nor activation of Raf-1 occurred following β1 engagement in monocytes (18). Recent studies have suggested the existence of unidentified MEK activators that may not have been detected in these studies (43). Further studies are required to evaluate the upstream activation pathway leading to MAP kinase activation following β1 integrin engagement in cells of monocytic lineage.

Although integrin ligation by antibody without aggregation induces phosphorylation and activation of p42 ERK-2, cross-linking consistently induces the most phosphorylation, ERK activation, and procoagulant response (Figs. 1 and 3). In addition, clustering by either adhesion-inhibiting antibodies as well as non-inhibitory antibodies induced comparable effects. These findings are consistent with previous reports demonstrating the need to aggregate integrin receptors in monocytes as well as in other cell types to achieve maximal tyrosine phosphorylation and gene expression (16, 44, 45). Considered together, the findings are consistent with previous reports demonstrating how inflammatory and anti-inflammatory mediator molecules acting via this or other signaling cascades might interact with this activated pathway to modulate the inflammatory response.

The present study is the first to describe a contributory role for the ERK pathway in the induction of adhesion-dependent inflammatory response in cells of monocyte/macrophage lineage. Since endothelial cell adhesion of monocytes via engagement of surface integrins is an early event in the mobilization of cells to sites of inflammation, it will be of interest to discern how inflammatory and antiinflammatory mediator molecules acting via this or other signaling cascades might interact with this activated pathway to modulate the inflammatory response.

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