Phosphorylation of vitronectin (Vn) by casein kinase II was previously shown to occur at Thr\(^{50}\) and Thr\(^{57}\) and to augment a major physiological function of vitronectin-cell adhesion and spreading. Here we show that this phosphorylation increases cell adhesion via the \(\alpha_5\beta_1\) integrin (not via the \(\alpha_6\beta_1\) integrin), suggesting that \(\alpha_5\beta_1\) differs from \(\alpha_6\beta_1\) in its biorecognition profile. Although both the phospho (CK2-PVn) and non-phospho (Vn) analogs of vitronectin (simulated by mutants Vn(T50E,T57E), Vn(T50A,T57A), respectively) trigger the \(\alpha_5\beta_1\) integrin as well as the \(\alpha_6\beta_1\) integrins, and equally activate the ERK pathway, these two forms are different in their activation of the focal adhesion kinase/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) pathway. Specifically, we show (i) that, upon exposure of cells to Vn/CK2-PVn, their PKB activation depends on the availability of the \(\alpha_5\beta_1\) integrin on their surface; (ii) that upon adhesion of the \(\beta_3\)-transfected cells onto the CK2-PVn, the extent of PKB activation coincides with the percentage adhesion of these cells, and (iii) that both the PKB activation and the elevation in the adhesion of these cells is PI3K-dependent. The occurrence of a cell surface receptor that specifically distinguishes between a phosphorylated and a non-phosphorylated analog of Vn, together with the fact that it preferentially activates different integrins (27, 31, 32). Several of these integrins, e.g. \(\alpha_5\beta_1\), \(\alpha_6\beta_1\), \(\alpha_6\beta_3\), \(\alpha_5\beta_3\), and \(\alpha_6\beta_3\) and the platelet-specific \(\alpha_{IIb}\beta_3\) integrin, are known to recognize and bind Vn. It is well known that cell adhesion is a complex process that was shown to involve an activation of several Vn receptors and a variety of intra-cellular signaling pathways. For example, the focal adhesion kinase (FAK) was shown to play a central role in mediating the signal from integrins (33). It does so by its autophosphorylation on Tyr\(^{397}\) upon integrin stimulation. This autophosphorylation leads to the recruitment and activation of intra-cellular mediators such as PI3K, as well as the Src family kinases, by an interaction of their SH2 domain with the auto-phosphorylated Tyr\(^{397}\) residue. The PI3K binding to Tyr\(^{397}\) leads to activation of PKB, whereas the Src family of kinases further phosphorylates FAK on Tyr\(^{325}\) leading to the recruitment of additional signaling molecules that bring about an activation of the ERK pathway (31–38).

We have previously shown that Vn can be functionally modulated by extra-cellular phosphorylation, making use of the kinase co-substrate ATP found at micromolar levels in the exterior of cells (39). For example PKA, released from platelets upon their physiological stimulation with thrombin (40–42), selectively phosphorylates Vn, and, as a consequence of this phosphorylation, it reduces its grip on plasminogen activator inhibitor-1 (43). Similarly, PKC phosphorylation of Vn was shown to attenuate its cleavage by plasmin (44). Several laboratories have shown the occurrence of an extra-cellular CK2 activity on a variety of cells. These include epithelial cells (45, 46), neutrophils (47, 48), platelets (49, 50), and endothelial cells (51–53). Subsequently, we showed that Vn is a substrate for CK2, which phosphorylates Vn at Thr\(^{50}\) and Thr\(^{57}\). Furthermore, we found that this phosphorylation significantly enhances the adhesion and spreading of bovine aorta endothelial cells (BAEC), presumably because the phosphorylated Vn has a higher affinity for \(\alpha_5\beta_1\) (54).

One of the major obstacles in revealing the mechanism of action of CK2-phosphorylated Vn originates from the well known fact that Vn (like other adhesion proteins) can bind to several integrins, including the specific Vn-binding integrin, \(\alpha_5\beta_1\), and that this family of integrins can, in turn, activate different intra-cellular pathways. Here we extend our studies on the consequences of the CK2 phosphorylation of Vn and...
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show that the enhanced cell adhesion involves αvβ5 (but not αvβ3). Furthermore, we show that this enhanced adhesion coincides with a preferential activation of the FAK/PI3K/PKB cascade, rather than the ERK signaling pathway.

**EXPERIMENTAL PROCEDURES**

**Chemicals, Materials, and Enzymes**—The following materials were purchased from the commercial sources: [35S]methionine (Amersham Pharmacia Biotech); nitrocellulose membranes (Schleicher & Schuell); restriction enzymes (Roche Molecular Biochemicals or Life Technologies, Inc.); T70 DNA polymerase (Promega).

**Antibodies**—Monoclonal antibodies against the integrin receptor αvβ5 (P1F6), against αvβ3 (LM609), and against the β3 integrin receptor (MAB 1974) were obtained from Chemicon. Monoclonal antibodies directed against the integrin receptor α3 (a and b) were from Serotec. Monoclonal antibodies against active ERK, JNK, and p38 MAPK were from Sigma Chemical Co. Monoclonal antibodies against phospho-tyrosine (PY99) were from New England Biolabs. 

**Tissue Cultures**—HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) heat-inactivated fetal calf serum and g/ml Fungizone (Life Technologies, Inc.) to contain 25000 cells/ml. H1299 cells were grown in RPMI supplemented with 10% (v/v) heat-inactivated fetal calf serum and g/ml g/ml hemoglobin (0.5 mg/ml). The cells were plated at a density 3 10^5 cells/ml. An aliquot of this suspension was added to the Vn-coated wells, and the remainder of the suspension was added to the non-Vn-coated wells. For some experiments, we used BAEC cells (24-well plates) that were plated at a density 0.5 10^6 cells/ml. The cells were incubated for 1.5 h at 22 °C in a shaking incubator (200 rpm) and then washed three times with 1 ml of PBS containing 1% bovine serum albumin and 0.02% sodium azide using a pipette. After the last wash, the samples were boiled in Laemmli’s sample buffer and subjected to SDS-PAGE. The gels were transferred to nitrocellulose membranes and blotted either with antibodies against phosphotyrosine (PY99, to detect phosphorylated FAK), or with antibodies against FAK (to determine the total FAK as a reference value) in each lane.

**RESULTS**

**Comparing the Adhesion of αvβ3- and αvβ5-bearing Cells in Their Response to Vn and to CK2-phosphorylated Vn**—We have previously shown (54) that the CK2 phosphorylation of Vn results in a significant enhancement of BAEC cell adhesion (~2.5-fold, average of three experiments), as indicated by the number of cells that adhere to increasing concentrations of immobilized Vn. We also showed that the effect of the CK2 phosphorylation could be reproduced with a mutant Vn(T50E,T57E) (a close analog of CK2-PVn representing the phospho form of Vn), when compared with Vn(T50A,T57A) (a close analog of Vn representing the non-phospho form of Vn). In the course of our studies we found that BAEC cells do not express αvβ5 (a characteristic binding receptor for Vn (55)); therefore, we considered the possibility that this integrin might be involved in a response to CK2-PVn and that this expression was not detected by the method we used HeLa cells (Fig. 1A) and H1299 cells (Fig. 1B), whose adhesion to Vn was found to be mediated mainly by αvβ5. In both cases we found an efficient inhibition of cell adhesion by anti-αvβ5, but a minor inhibition by anti-αvβ3. A similar inhibition of cell adhesion by both antibodies was also obtained with Vn(T50A,T57A) (not shown), raising the possibility that the
adhesion of these cells to both forms of Vn is mediated by $\alpha_3\beta_3$. In line with this finding, the adhesion profile of HeLa as well as H1299 cells to immobilized Vn(T50E,T57E) was found to be essentially identical to their adhesion to Vn(T50A,T57A) (Fig. 1, C and D). In this context it should be noted that (i) the same adsorption profile of the cells was obtained whether Vn(T50E,T57E) or Vn(T50A,T57A) was used as a substratum (54) and (ii) in all experiments comparing Vn(T50A,T57A) with Vn(T50E,T57E) we ran a similar experiment with wild type r-Vn and showed that, within experimental error, it was identical to Vn(T50A,T57A).

**Cells Containing $\alpha_3\beta_3$ Exhibit an Enhanced Cell Adhesion upon Exposure to CK2-PVn**—The results presented above, together with our previous findings with BAEC (54), imply that the enhanced cell adhesion onto CK2-PVn is mediated by the $\alpha_3\beta_3$ receptor. To confirm this suggestion we endowed H1299 cells (which do not exhibit an enhanced cell adhesion in response to CK2-PVn) with a capability to exhibit an enhanced cell adhesion onto Vn(T50E,T57E) and thus to “discriminate” between the phospho- and non-phospho forms of Vn. This was achieved by transfecting H1299 cells with the $\beta_3$ subunit. Isolated clones of H1299 cells overexpressing $\alpha_3\beta_3$ that were identified by immunoblotting with anti-$\beta_3$, and subsequently characterized by FACS analysis with anti- $\alpha_3\beta_3$ (Fig. 2, A and B), were shown to contain high amounts of the $\alpha_3\beta_3$ integrin on their surface. Quantitation of the FACS analysis indicated that the $\beta_3$-transfected clones we used contained up to 7-fold more $\alpha_3\beta_3$ than the control vector-transfected clones, whereas the amounts of the $\alpha_3$ and of a non-relevant $\alpha_3$ integrin were very similar to the control. In addition, we observed a 3-fold reduction of $\alpha_3\beta_3$ in the $\beta_3$-transfected clone, presumably due to competition between $\beta_3$ and the excess of $\beta_3$ for the limited amount of their common partner, the $\beta_3$ subunit.

The involvement of $\alpha_3\beta_3$ (but not $\alpha_3\beta_5$) in the enhanced cell adhesion is best illustrated in Fig. 3, which shows that the adhesion of vector-transfected H1299 cells is blocked by anti-$\alpha_3\beta_3$ (Fig. 3A), whereas the adhesion of $\beta_3$-transfected H1299 cells is blocked by anti-$\alpha_3\beta_5$ but not by anti-$\alpha_3\beta_3$ (B). In line with these findings, the vector-transfected H1299 cells do not discern Vn(T50E,T57E) from Vn(T50A,T57A), whereas cells overexpressing the $\beta_3$ subunit exhibit an ability to enhance cell adhesion on the Vn(T50E,T57E) mutant (compare Fig. 3C with Fig. 3D). It should be noted that the occurrence of a relationship between the integrin content of cells, their adhesion, and the ensuing intracellular signaling triggered by Vn were also observed with two additional $\beta_3$-transfected clones (not shown).

**An ERK Activation Cannot Account for the Enhanced Cell Adhesion Observed with CK2-PVn**—Following the identification of $\alpha_3\beta_3$ as a CK2-PVn-specific mediator of the enhanced...
adhesion obtained with this phosphorylation, we attempted to identify an intra-cellular signaling pathway that might be responsible for this enhancement. Because the activation of ERKs in response to the stimulation of cells by ECM proteins was already established (31–38), we first examined the pattern of ERK activation in the stable clones of H1299 cells as determined by immunoblot. Soluble fractions obtained from extracts of H1299 stably transfected with the cDNA of αvβ3 integrin from extracts of H1299 stably transfected with the cDNA of αvβ3 integrin, or with vector alone (pcDNA3), were analyzed by immunoblotting with anti-β3 monoclonal antibodies. Lane 1, non-transfected cells; lanes 2 and 3, two different vector-transfected clones; lanes 4 and 5, two different β3-transfected clones. B, quantitation of the integrins expressed in the stable clones of H1299 cells by FACS analysis. Cells were incubated with monoclonal antibodies directed against the indicated integrin, followed by incubation with the secondary antibodies, FITC-conjugated goat anti-mouse IgG (heavy line). Control cells were incubated only with the secondary antibody (light line).

The Increased Activation of the PKB Pathway Can Account for the Enhanced Cell Adhesion Mediated by αvβ3—Because we found that the activation of ERK cannot account for the enhanced cell adhesion, we looked into other signaling pathways such as the JNK, p38 MAPK, and PKB pathways that were previously shown to be activated by Vn-binding integrins. Although no adhesion-triggered activation of JNK and p38 MAPK was detected in the various clones we used (data not shown), we found that the activation of PKB in the β3-transfected cells (Fig. 5) led to a significantly enhanced activation of this kinase, in comparison to the very low PKB activation in the vector-transfected cells. These results suggested to us that the activation of PKB depends on the availability of the αvβ3 integrin. As such, the extent of PKB activation in the β3-transfected cells correlates well with the extent of enhanced cell adhesion onto CK2-PVn. This was demonstrated with β3-transfected cells that were plated on Vn(T50E,T57E), whose enhanced adhesion resulted in an increased PKB activation (∼30-fold over the PDL control), whereas the PKB activation obtained in cells plated onto Vn(T50A,T57A) was found to be only 18-fold over the control (Fig. 5C).

PI3K Is Essential for the Promotion of Cell Adhesion and for the Activation of PKB—PKB was recently implicated as an important downstream target for PI3K (56). To determine whether the PKB activation in our system requires the activation of PI3K (which precedes PKB in several signal transduction processes (cf. Scheme 1), we treated β3-transfected cells with wortmannin (a PI3K inhibitor) prior to their stimulation by adhesion to Vn(T50E,T57E). Indeed, wortmannin prevents PKB activation (Fig. 6A), presumably through a PI3K inhibition, indicating that the enhanced adhesion mediated by αvβ3 transmits the signal to PKB via PI3K. In line with this result, the enhanced adhesion of the β3-transfected cells was reduced by preincubation with wortmannin (before allowing the cells to adhere) (Fig. 6B) or with another PI3K inhibitor LY294002 (Fig. 6C). As expected, these two inhibitors blocked cell adhesion onto both the phospho- and the non-phospho forms of Vn, because PKB is activated by both forms of Vn. However, the reduction in the elevation in cell adhesion onto CK2-PVn over Vn, illustrated by using PI3K inhibitors, clearly indicates the involvement of this pathway in elevating cell adhesion on CK2-PVn. The specific involvement of the PI3K-PKB pathway in the adhesion of cells onto the phospho and the non-phospho forms of Vn was supported by our finding that the MEK inhibitor PD98059 does not inhibit the cell adhesion onto these two Vns (Fig. 6D). In that context it should be noted that, in our experiments, the PKB activation occurs within 5–10 min after plating the cells (the cells are attached but not spread), whereas the adhesion of the cells proceeds for 30 min when the cells are already adhered and spread. This observation sets the stage for a detailed study aimed at the identification of the sequence of events that lead from cell adhesion to cell spreading, namely at the elucidation of the mechanism by which downstream mediators of PKB influence the cell-spreading process.

In conclusion, it is evident from our results (i) that the PKB activation (which occurs upon exposure of cells to Vn/CK2-PVn) depends on the availability of the αvβ3 integrin on the surface of the cells; (ii) that the extent of PKB activation (that takes place upon exposure of the β3-transfected cells to CK2-PVn) coincides with the specific enhanced adhesion of these cells upon their binding to CK2-PVn; and (iii) that both the PKB activation and the subsequent enhanced adhesion of the cells are PI3K-dependent, because the inhibition of PI3K (upstream of PKB) prevents the PKB activation and reduces cell adhesion (Scheme 1).

Cells Containing αvβ3 and αvβ3 Differ in Their FAK Phosphorylation Pattern upon Their Adhesion onto Phospho and Non-phospho Forms of Vn—As mentioned above, there is a significant difference in the intensity of the PKB activation upon exposure of β3-transfected cells to the phospho and the non-phospho forms of Vn (Fig. 5). To account for this difference in intensity (shown here to be PI3K-dependent (Fig. 6A)), we...
compared their FAK phosphorylation pattern, i.e. the possible activation of an upstream kinase in this pathway. It is well known that the phosphorylation of FAK is an early event detected in response to integrin stimulation (33). Upon this stimulation, FAK is autophosphorylated on Tyr397, creating a high affinity binding site for a variety of kinases containing an SH2 domain, including the PI3K and Src family kinases. Src further phosphorylates FAK on Tyr925, leading to the recruitment of GRB2, which is known to activate the ERK pathway (38). Therefore, we monitored the FAK phosphorylation upon attachment of vector/b3-transfected cells onto the r-Vns mentioned above. As seen in Fig. 7, the FAK phosphorylation is different in these two cell lines. Although there is a gradual activation of FAK that peaks after 20 min in the vector-transfected clone (Fig. 7A), the FAK phosphorylation in b3-transfected cells is weaker and transient. It peaks after 5–10 min and declines thereafter (Fig. 7B). The time course of FAK phosphorylation in the b3-transfected cells coincides with that of PKB activation (Fig. 5B). Moreover, although no differences in FAK phosphorylation were observed when vector-transfected cells were plated either on Vn(T50A,T57A) or on Vn(T50E,T57E) (Fig. 7, B and C), a preferential increase in FAK phosphorylation was observed when b3-transfected cells were plated on the Vn(T50E,T57E) mutant (5 min, Fig. 7B). Although a small increase, this signal is amplified, and a better reflection of it is viewed in the differences observed in the downstream kinase PKB (Fig. 5). The FAK phosphorylation in the vector-transfected cells is significantly more intense at the peak of the response (20–30 min). This may suggest that in the vector-transfected cells (α3β3) another kinase may further phosphorylate FAK, whereas in the b3-transfected cells (α3β3) FAK autophosphorylation brings about the association with PI3K, which does not further phosphorylate FAK but, rather, specifically activates the PKB pathway. We conclude that the extra-cellular stimulation by CK2-PVn (as represented by Vn(T50E,T57E)) is transmitted via αvβ3 and that the PI3K pathway is involved in the enhanced cell adhesion.

**DISCUSSION**

Intra-cellular protein phosphorylation is now well established as a central regulatory mechanism. In the last few years, several reports provided evidence for the occurrence of protein kinases outside the cell, raising the possibility that protein phosphorylation may also regulate extra-cellular processes (40, 41, 45–48). This possibility was supported by the identification of specific target substrates for the kinases in the cell exterior. Some reports further indicated that the physiological function of such specific substrates is modulated upon their phosphorylation (for a review see Ref. 42). For example, it was shown that Vn is functionally modulated by PKA, a kinase released from platelets upon their physiological stimulation with thrombin (40–42). Similarly, a PKC phosphorylation of Vn was shown to attenuate its cleavage by plasmin (44).

In addition to PKA and PKC, Vn was recently shown to be a substrate for CK2, which was found to single out and selectively phosphorylate Vn at Thr50 and Thr57 to bring about a significant enhancement of one of Vn’s well known physiological functions: cell adhesion and spreading (54). The clinical importance of this modulation is evident in view of the fact that invasive metastasis involves an enhanced adhesion of tumor cells to the ECM (6) by binding to integrins, in particular α3β3. In fact, this integrin has been implicated in the acquisition of...
metastatic invasiveness (57). In melanoma, for example, the expression of $\alpha_v\beta_3$ was shown to correlate with invasiveness (58) and with tumorigenic capacity (57, 59). In the case of $\alpha_v$, the specificity in the recognition of its CK2-phosphorylated form may have a special importance in cancer, because $\alpha_v$ seems to be an important ligand in the $\alpha_v\beta_3$-mediated adhesion of tumor cells. In line with this fact, human melanoma cells derived from lymphatic metastases were shown to use $\alpha_v\beta_3$ to adhere to lymph node $\alpha_v$ (7), in a $\alpha_v$-mediated manner, as indicated by the fact that the replacement of $\alpha_v$ by fibronectin had no effect on invasion (60).

A major implication of the findings presented in this report is that the CK2 phosphorylation of $\alpha_v$ enhances cell adhesion via $\alpha_v\beta_3$ but not via $\alpha_v\beta_5$. This can be deduced from our finding that cells that adhere mostly via $\alpha_v\beta_5$ (e.g. HeLa cells, or the H1299 lung carcinoma cells) do not distinguish between the mutant Vn(T50E,T57E) and Vn(T50A,T57A). Furthermore, we report here that the enhanced cell adhesion can be quantitatively accounted for by assuming that the integrin $\alpha_v\beta_3$ alone is involved in the preferential recognition of the $\alpha_v$ analog Vn(T50E,T57E), i.e. in the specific response to CK2-phosphorylated Vn. In line with this conclusion the H1299 lung carcinoma cells (whose adhesion to Vn is mediated by $\alpha_v\beta_3$) are not able to

![Fig. 4. ERK activation triggered by r-Vns in the stably transfected H1299 cells.](image)

![Fig. 5. PKB activation triggered by r-Vns in the stably transfected H1299 cells.](image)

![Scheme 1. Schematic presentation of the ERK and PKB signaling pathways in response to integrin stimulation.](image)
FIG. 6. Inhibition of PKB activation by inhibitors of PI3K also inhibits cell adhesion. A, the PKB activation triggered by Vn(T50E,T57E) in the β3-transfected H1299 cells is inhibited by wortmannin. Plates were coated with Vn(T50E,T57E) (A, lanes 2) or with PDL (A, lanes 1). The cells were preincubated with dimethyl sulfoxide (DMSO) (0.1%), or with wortmannin (100 nM/0.1% DMSO), for 15 min (37°C) with gentle shaking, then were plated on top of the coated plates for 10 min. Adhered cells were harvested in RIPA buffer, and the soluble fractions were collected after centrifugation. The protein concentration was determined and equal amounts of protein were loaded on SDS-PAGE. The gels were transferred to nitrocellulose membranes and blotted with anti-active PKB or with anti-total PKB.

B, the adhesion of β3-transfected H1299 cells to Vn(T50E,T57E) is inhibited by wortmannin. Polystyrene plates were coated with increasing concentrations of the two r-Vns: Vn(T50A,T57A) (○, ▲) and Vn(T50E,T57E) (□, ●). H1299 cells stably transfected with the β3 subunit were labeled with [35S]Met. The labeled cells were preincubated with wortmannin (100 nM/0.1% DMSO; filled symbols) or with DMSO (0.1%; empty symbols) for 15 min (37°C) with gentle shaking, then plated on top of the two r-Vns. The cell adhesion after 30 min was determined by counting the residual radioactivity after extensive washing as described under “Experimental Procedures.” C, the adhesion of β3-transfected H1299 cells to Vn(T50E,T57E) is inhibited by LY294002. Polystyrene plates were coated with increasing concentrations of the two r-Vns: Vn(T50A,T57A) (○, ▲) and Vn(T50E,T57E) (□, ●). H1299 cells stably transfected with the β3 subunit were labeled with [35S]Met. The labeled cells were preincubated with LY294002 (25 μM/0.1% DMSO; filled symbols) or with DMSO (0.1%; empty symbols) for 15 min (37°C) with gentle shaking then plated on top of the two r-Vns. The cell adhesion after 30 min was determined by counting the residual radioactivity after extensive washing as described under “Experimental Procedures.” D, the adhesion of β3-transfected H1299 cells to Vn(T50E,T57E) is not inhibited by PD98059. Polystyrene plates were coated with increasing concentrations of the two r-Vns: Vn(T50A,T57A) (○, ▲) and Vn(T50E,T57E) (□, ●). H1299 cells stably transfected with the β3 subunit were labeled with [35S]Met. The labeled cells were preincubated with PD98059 (25 μM/0.1% DMSO; filled symbols) or with DMSO (0.1%; empty symbols) for 15 min (37°C) with gentle shaking then plated on top of the two r-Vns. The cell adhesion after 30 min was determined by counting the residual radioactivity after extensive washing as described under “Experimental Procedures.”
discriminate between Vn(T50E,T57E) and Vn(T50A,T57A) but gain the ability to discriminate between these two mutants upon their stable transfection with the $\alpha_5\beta_3$ integrin subunit.

In view of our finding that the enhanced cell adhesion onto Vn(T50E,T57E) is mediated by $\alpha_5\beta_3$ and our previous observation that this enhancement is due to an increased affinity toward this integrin, we undertook to identify the signaling pathway that is involved in this increased affinity. Several signaling cascades were previously shown to be activated by the integrin family of receptors (31–34, 36–38, 61, 62). Having in our hands cells that use $\alpha_5\beta_3$ to adhere onto Vn, and essentially identical companion cells that use $\alpha_5\beta_2$ to adhere to this integrin ligand, enabled us to identify a signaling pathway, which is differentially activated upon adhesion of these cells to CK2-phosphorylated and non-phosphorylated Vn analogs. Specifically, we were able to show that the phospho and non-phospho forms of Vn trigger both $\alpha_5\beta_3$ and $\alpha_5\beta_2$, leading to a similar activation of ERK. These results suggest that the activation of ERK occurs via the $\alpha$ subunit (61), which has not been modified in these cells. The fact that the activation of ERK is not influenced by the introduction to the $\beta_3$ subunit supports this suggestion. In contrast, the PKB activation seems to depend on the availability of the $\beta_3$ subunit, and therefore, is preferentially activated by the phospho form of Vn. We presume that this enhanced activation of PKB, which is $\alpha_5\beta_3$- and PI3K-dependent, results in the enhanced cell adhesion by the CK2-PVn analog (Vn(T50E,T57E)).

Based on the results presented here, we suggest that although both $\alpha_5\beta_3$ and $\alpha_5\beta_2$ share common structural elements that recognize and bind equally well the core protein shared by Vn and PVn, $\alpha_5\beta_3$ contains additional recognition elements that bind the two phosphopeptide groups specifically introduced in Vn by its CK2 phosphorylation. Specifically, this result raises the possibility that the ligand binding site of $\alpha_5\beta_3$ possesses recognition elements to CK2-PVn that are not present in $\alpha_5\beta_2$. This suggestion, which is supported by additional experimental evidence using a set of RGD-containing peptides as inhibitors, 4 can account for the distinct behavior of the $\alpha_5\beta_3$ and $\alpha_5\beta_2$ integrins and specifically for the $\alpha_5\beta_3$-mediated enhanced activation of the PI3K/PKB pathway that correlates with the increased cell adhesion.

One of the important messages reported here lies in the fact that it identifies two intracellular signaling pathways that are unequally activated upon binding of Vn and CK2-PVn, at least to the cells we tested in this study. Both pathways (one functioning via $\alpha_5\beta_3$ and $\alpha_5\beta_2$ and one via $\alpha_5\beta_3$ (Scheme 1)) are activated upon adhesion of the cells onto Vns. However, although the activation of ERK (triggered by both $\alpha_5\beta_3$ and $\alpha_5\beta_2$) was not modified upon cell adhesion onto CK2-PVn, the activation of PKB (triggered by $\alpha_5\beta_3$ but not by $\alpha_5\beta_2$) is elevated upon adhesion to CK2-PVn. It is this elevation that is correlated with the enhanced cell adhesion. Therefore, we propose that the PI3K/PKB pathway (and not the ERK pathway) reflects the $\alpha_5\beta_3$-mediated enhanced cell adhesion (Scheme 1). In line with this proposal, we found that blocking the activation of ERK by an MEK inhibitor did not have an effect on cell adhesion. In contrast, the blocking of PKB by PI3K inhibitors reduced cell adhesion.

Taken together, the results presented here together with our results reported earlier (54) indicate the occurrence of a cell surface receptor ($\alpha_5\beta_3$) and an intracellular signaling pathway that distinguish between a CK2-phosphorylated and a non-phosphorylated form of Vn. These results are based on a CK2-phospho and a non-phospho form of Vn, two mutant analogs of Vn, three different cell lines, and four independent cell clones. We believe that these findings indicate that the extra-cellular phosphorylation of Vn by CK2 may well be a physiological process with a distinct regulatory role in the control of cell adhesion and spreading.

Acknowledgments—We thank Dr. Iris Schwartz for stimulating discussions and Shoshana Lichter and Tamar Hanoch for technical assistance.

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The CK2 Phosphorylation of Vitronectin: PROMOTION OF CELL ADHESION VIA THE αβ3-PHOSPHATIDYLINOSITOL 3-KINASE PATHWAY
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J. Biol. Chem. 2001, 276:16998-17006.
doi: 10.1074/jbc.M003766200 originally published online February 23, 2001

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