Integrin-mediated cell attachment and growth factor stimulation often act synergistically on cell proliferation, differentiation, migration, and survival. Some of these synergistic effects depend on the physical interaction of integrins with growth factor receptors. Here we examine the nature of the physical interaction between the $\alpha_\beta_3$ integrin and two receptor tyrosine kinases (RTKs), the platelet-derived growth factor receptor $\beta$ (PDGF-R$\beta$) and the vascular endothelial growth factor receptor 2 (VEGF-R2, also known as KDR and flk-1). Both of these RTKs associate with the $\alpha_\beta_3$, integrin but do not associate with $\beta_3$, integrins. Furthermore, growth factor stimulation of these RTKs promotes increased cell proliferation and migration when cells are attached to the $\alpha_\beta_3$ ligand, vitronectin. We show that $\alpha_\beta_3$ in which the $\beta_3$ cytoplasmic domain is deleted or replaced with the $\beta_3$ cytoplasmic domain communoprecipitates with PDGF-R$\beta$ and VEGF-R2. The $\beta_3$ extracellular domain alone was sufficient for the PDGF-R$\beta$ association whereas the VEGF-R2 association required the presence of the $\alpha_3$ subunit. Activation of the RTKs by their ligands was not required for them to associate with the integrin. Cell migration to PDGF was enhanced in the cells transfected with the chimeric subunit containing the $\beta_3$ extracellular domain but not when that domain came from the $\beta_3$ subunit. These results show that the interactions that lead to the association of the $\alpha_\beta_3$ integrin with PDGF-R$\beta$ and VEGF-R2 and enhancement of RTK activity take place outside the cell.

Integrins mediate cell adhesion to extracellular matrix proteins and to other cells. Integrins also initiate intracellular signaling events that control cell shape, migration, proliferation, differentiation, and survival (1, 2). Many of the intracellular molecules that mediate integrin signaling also participate in signaling events initiated by soluble growth factors and their transmembrane receptors. Examples of these intracellular molecules include protein kinases, such as c-Src; small GTPases, such as Ras and Rac; phosphatidylinositol 3-kinase; the protein-tyrosine phosphatase SHP-2; and adaptor molecules, such as Shc (3–5).

Another form of cross-talk between integrins and growth factor receptors involves physical interaction between the two classes of proteins and potentiating of growth factor signals upon extracellular matrix binding of the interacting integrin (2). Integrin interactions with receptor tyrosine kinases (RTKs)$\dagger$ have been studied in some detail. RTKs are transmembrane proteins with an extracellular domain that binds the ligand and an intracellular kinase domain that becomes autophosphorylated upon binding of the ligand to the receptor. Cell adhesion to fibronectin or to antibodies against the $\beta_3$ integrin subunit causes autophosphorylation of certain RTKs (6, 7) and shifts their localization to focal adhesions (8), even in the absence of the growth factor ligand.

Some RTKs interact physically with integrins. The EGF receptor forms a complex with $\beta_3$ integrins after cells attach to fibronectin (7). Phosphorylated PDGF-R$\beta$ coprecipitates with $\alpha_\beta_3$ but not with $\beta_3$ integrins (9, 10), similar to the insulin receptor (9, 11) and the VEGF-R2 (12). Upon stimulation, all of these $\alpha_\beta_3$-associated growth factor receptors induce increased proliferation and migration in cells attached to the $\alpha_\beta_3$ ligand vitronectin. Hence, RTKs selectively interact with certain integrins, and these interactions result in a synergistic signaling effect. However, little is known about the mechanism of the integrin-RTK interaction.

The goal of this study was to localize the sites of the $\alpha_\beta_3$ integrin that interact with PDGF-R$\beta$ and VEGF-R2. We show that the extracellular domain of the $\beta_3$ subunit mediates the interaction with PDGF-R$\beta$ and VEGF-R2, whereas the cytoplasmic and transmembrane regions of the $\beta_3$ subunit are dispensable. We also find that binding of the growth factor ligand by these RTKs and the resultant phosphorylation of the receptor are not required for the $\alpha_\beta_3$ interaction. VEGF-R2 requires the $\alpha_3$ subunit for efficient association with $\alpha_\beta_3$, whereas PDGF-R$\beta$ does not have this requirement.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Growth Factor—**Polyclonal rabbit antibodies prepared against the cytoplasmic peptides of the $\beta_3$ and $\beta_3$ integrin subunits and against purified $\alpha_\beta_3$ have been described (11, 13). Rabbit antibodies against $\alpha_3$ PDGF-R$\beta$, and VEGF-R2 were from Santa Cruz Biotechnology, rabbit antibodies against the $\beta_3$ integrin subunit were from Chemicon, and the peroxidase-labeled anti-phosphotyrosine antibody, PY20-horseradish peroxidase, was from Transduction Laboratories. Human PDGF-BB and mouse and human VEGF were obtained

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* This work was supported by Grant CA67224 and Cancer Center Support Grant CA30199 from the NCI, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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$\dagger$ The abbreviations used are: RTKs, receptor tyrosine kinases; PDGF-R$\beta$, platelet-derived growth factor receptor $\beta$; VEGF-R2, vascular endothelial growth factor receptor 2; EGF, epidermal growth factor; PAE cells, porcine aortic endothelial cells; CHO, Chinese hamster ovary.
from R&D Systems. Horseradish peroxidase-conjugated Protein A was purchased from Sigma.

**Constructs—**cDNAs were constructed in the pcDNA3 vector (Invitrogen). Mouse VEGF-R2 (flk-1) cDNA was provided by Dr. Georg Breier, and the human PDGF-R chain cDNA was provided by Dr. Carl-Henrik Heldin. The \(\beta_3\) cytoplasmic domain deletion \((\beta_3\text{-acyto})\) construct lacks the cytoplasmic tail except for the juxtamembrane heptapeptide KLIILTIH.

**Immunoprecipitation and Immunoblotting—**After transfection (24 h), cells were starved overnight in Dulbecco’s modified Eagle’s medium (DMEM) without supplements, except that for experiments with VEGF-R2, 0.5% fetal calf serum was added. For some studies, cells were treated for 1 h at 37 °C with 30 \(\mu\)g/ml AG1296 (Calbiochem), a specific inhibitor of the PDGF-receptor kinases (17). Cells were stimulated as indicated with 20 ng/ml PDGF or 50 ng/ml VEGF for 5 min at 37 °C, washed once with ice-cold phosphate-buffered saline, and lysed for 15 min in Nonidet P-40 buffer (1% Nonidet P-40, 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 2 mM sodium pyruvate, glutamine, antibiotics (all from Irvine Scientific), and non-essential amino acids (Life Technologies, Inc.). Cells were transfected with FuGENE 6 (Roche) according to the vendor’s protocol. The \(\alpha_{v}/\beta_3\) heterodimeric cells were disrupted by incubation with 10 mM EDTA in phosphate-buffered saline for 5 min at 37 °C (15, 16).

**RESULTS**

**PDGF-R\(\beta_3\) and VEGF-R2 are Associated with the \(\beta_3\) Integrin Subunit—**Previous studies have shown that PDGF-R\(\beta_3\) (9, 10) and VEGF-R2 (12) associate with the \(\alpha_v\) integrin but not with \(\beta_1\) integrins. The association was detected after cells were stimulated with the respective growth factor. We have studied these interactions in PAE and CHO cells transfected with integrin subunits and PDGF-R\(\beta_3\) or VEGF-R2. Both cell lines express only minor amounts of endogenous \(\beta_3\) and PDGF-R\(\beta_3\) (Fig. 2A) and no detectable VEGF-R2 (Fig. 2B). These cells were transfected with cDNAs of \(\alpha_v\) and \(\beta_3\) together with PDGF-R\(\beta_3\) (PAE) or with VEGF-R2 (CHO). The \(\beta_3\) integrin was precipitated with antibodies directed against its cytoplasmic tail, and the immunoprecipitates were probed for the presence of the RTKs by immunoblotting. PDGF-R\(\beta_3\) (Fig. 2A) and VEGF-R2 (Fig. 2B) could only be detected in anti-\(\beta_3\) precipitates from cells that had been transfected with the \(\alpha_v\) and \(\beta_3\) subunits. Because our anti-\(\beta_3\) antibody reacts with \(\beta_3\) of a number of species (9, 18), it is likely that the level of \(\beta_3\) in CHO and PAE cells is too low for the endogenous \(\alpha_v\) and \(\beta_3\) subunits to coprecipitate detectable amounts of the RTKs. Antibodies against purified \(\alpha_v\) coprecipitated the RTKs from the same cells as the anti-\(\beta_3\) (data not shown).

**Growth Factor Stimulation of RTKs Is Not Necessary for the Integrin Association—**Stimulation of cells with the respective growth factor promotes the association of PDGF-R\(\beta_3\) (9) and VEGF-R2 (12) with the \(\alpha_v\) integrin. We found that the amount of VEGF-R2 associated with the \(\beta_3\) integrin in CHO cells was similar, regardless of prior VEGF stimulation (Fig. 3A). Fig. 3B compares the phosphorylation level of VEGF-R2...
directly precipitated from starved and VEGF-stimulated cells. Only VEGF-R2 from the VEGF-stimulated cells was significantly phosphorylated. These results show that ligand binding and phosphorylation of VEGF-R2 are not required for the receptor to interact with \( \alpha_\beta_3 \) subunits in these transfected CHO cells.

The cells transfected with PDGF-R\( \beta \) and the integrin subunits showed strong phosphorylation of PDGF-R\( \beta \) even after 24 h of starvation. To assess whether phosphorylation of the PDGF-R\( \beta \) is required for its interaction with \( \alpha_\beta_3 \), we pretreated the transfected CHO cells with AG1296 (18), a specific inhibitor of PDGF-receptor kinase, to prevent receptor auto-phosphorylation. Equal amounts of PDGF-R\( \beta \) were associated with \( \alpha_\beta_3 \) (Fig. 3A) regardless of AG1296 treatment or PDGF stimulation, even though AG1296 prevented the PDGF-R\( \beta \) phosphorylation (Fig. 3B). We conclude that ligand binding and phosphorylation are not necessary for PDGF-R\( \beta \) and VEGF-R2 to interact with the \( \alpha_\beta_3 \) integrin, at least when the components of the system are expressed at high levels in cells.

**The \( \beta_3 \) Subunit Is Crucial for the Interaction of PDGF-R\( \beta \) and VEGF-R2 with the Integrin**—To study the site of the RTK-integrin interaction on the integrin, we first set out to identify the integrin subunit that mediates formation of the complex. RTKs were cotransfected with \( \alpha_\beta_3 \), both RTKs immunoprecipitated with \( \alpha_\beta_3 \) regardless of AG1296 treatment or PDGF stimulation. As shown in Fig. 4, this result agrees with previous results for VEGF-R2 (12), and it demonstrates that the \( \beta_3 \) subunit is essential for interaction with the RTKs.

To study the role of \( \alpha_\beta_3 \) in the formation of the RTK-integrin complex, we transfected PDGF-R\( \beta \) into CHO cells that overexpress the platelet integrin \( \alpha_{IIb} \beta_\lambda \). As shown in Fig. 5A, equal amounts of PDGF-R\( \beta \) were coprecipitated with both anti-\( \alpha_{IIb} \) and anti-\( \beta_\lambda \) antibodies. To rule out the possibility that \( \alpha_\beta_3 \) would play a role and that \( \alpha_{IIb} \) would substitute for it in the binding of PDGF-R\( \beta \), we disrupted part of the \( \alpha_{IIb} \beta_\lambda \) heterodimer with EDTA prior to immunoprecipitation. As shown in Fig. 5A,
fails to induce signaling cascades, focal contacts, or cell spread-

most of the cytoplasmic domain (Fig. 1). This mutant subunit

associate with only the

b subunit could be coprecipitated with anti-

CHO cells with a b tion of the a subfraction of the a lane 3). Similar to earlier reports, EDTA treatment only dis-

amount of PDGF-R

dissociation of some of the a b heterodimers did not alter the amount of PDGF-R recovered with the b subunit. However, disrupting the a b heterodimer drastically reduced the amount of PDGF-R recovered with the a b antibody (Fig. 5B, lane 3). Similar to earlier reports, EDTA treatment only disrupted a subfraction of the a b heterodimer (15, 16). These data show that the a subunit is not important for the interaction of the b integrins with PDGF-R.

In contrast to PDGF-R b, VEGF-R2 did not coprecipitate with the a b integrin. Neither antibodies against the a subunit nor against the b subunit coprecipitated VEGF-R2 from the a b-expressing CHO cells transfected with this RTK (Fig. 5C). To prove that this lack of coprecipitation is due to the low level of endogenous a, we transfected the a b-expressing CHO cells with a together with VEGF-R2 to obtain higher amounts of a b heterodimers. From these cells, VEGF-R2 could be coprecipitated with anti-b but not with anti-a. These data show that VEGF-R2 can only associate with the a b integrin, whereas PDGF-R b can form complexes with both a b and a b.

PDGF-R b Inhibits the Association of VEGF-R2 with the a b Integrin—As shown, the a b association of PDGF-R b requires only the b subunit, whereas association with VEGF-R2 requires both the a and b subunits. To address whether the RTKs have overlapping or distinct binding sites on a b, we overexpressed PDGF-R b to study the ability of VEGF-R2 to associate with a b. Cotransfecting a 4-fold excess of PDGF-R b prevented coimmunoprecipitation of VEGF-R2 with anti-a (Fig. 6A); only PDGF-R b was present in the b immunoprecipitates (Fig. 6B). Overexpressing VEGF-R2 did not inhibit the coimmunoprecipitation of PDGF-R b with a b (Fig. 6B) even when VEGF-R2 was expressed in 80-fold excess (data not shown). A difference in the binding affinities of the two RTKs for the integrin may explain this lack of inhibition by VEGF-R2.

The Extracellular Domain of b Mediates the RTK Interaction—To determine which part of the b subunit mediates the RTK interaction, we first generated a b subunit that lacks most of the cytoplasmic domain (Fig. 1). This mutant subunit fails to induce signaling cascades, focal contacts, or cell spread-

ing (19) but forms heterodimers with a, that can mediate cell adhesion to vitronectin. Immunoprecipitation from CHO cells transfected with wild-type b or its cytoplasmic deletion mu-

tant together with a, and VEGF-R2 yielded similar amounts of a b-associated VEGF-R2 regardless of the presence or absence of the b cytoplasmic domain (Fig. 7). No VEGF-R2 was coprecipitated without cotransfection of a b subunit, presumably reflecting an absence of endogenously expressed b subunit. lation—

Fig. 4. VEGF-R2 and PDGF-R b associate with the b subunit but not with the b subunit. CHO cells were transfected with a, and either the b (lanes 1 and 3) or b (lanes 2 and 4) subunit. In addition, they were transfected with PDGF-R b (lanes 1 and 2) or VEGF-R2 (lanes 3 and 4). Cell extracts were immunoprecipitated with antibodies against the cytoplasmic tail of the individual b subunits and analyzed for the presence of RTKs by immunoblotting.

Fig. 5. PDGF-R b, but not VEGF-R2, associates with the plate-
et integrin a b. A, a b-expressing CHO cells were transfected with the PDGF-R b and either treated with EDTA for 5 min at 37 C (lanes 1 and 3) or left untreated (lanes 2 and 4). Immunoprecipitates obtained with anti-b (lanes 1 and 2) or anti-a (lanes 3 and 4) antibody were analyzed for the presence of PDGF-R b by immunoblotting with the PY20 antibody. B, the same membrane as in A was stripped and analyzed for the amount of precipitated b subunit by reprobing with antibodies against purified VNR. C, a b-expressing CHO cells were transfected with VEGF-R2 alone or with a and VEGF-R2, and cell extracts were immunoprecipitated with anti-a b or b antibodies and probed for the presence of VEGF-R2 by immunoblotting.
indicate that the cytoplasmic domain of the various RTKs.

To confirm the result with the cytoplasmic domain deletion mutant and to study the role of the transmembrane domain, we took advantage of the fact that PDGF-Rβ and VEGF-R2 do not become associated with β1 integrins. Chimeric constructs of the β1 and β3 subunit in which the cytoplasmic and transmembrane domains came from one subunit and the extracellular domain from the other (Fig. 1) formed heterodimers with the αv subunit. This result was shown by immunoprecipitation with αv-specific antibodies from CHO cells transfected with αv and the various β-subunit constructs (data not shown). Both PDGF-Rβ and VEGF-R2 coprecipitated with the β3/1 chimera containing the β3 extracellular domain (Fig. 8). Antibodies against the cytoplasmic tail of the β1 subunit and antibodies against purified human αvβ3 both coprecipitated the RTKs equally well (Fig. 8 and data not shown). In contrast, the RTKs did not coprecipitate with the β1/3 chimera, which consists of the cytoplasmic and transmembrane regions of β3 and the extracellular domain of β1, or wild-type β1 (Fig. 8). The β1/3 chimera was precipitated with an antibody against the cytoplasmonic domain of β1. These data show that the extracellular domain of the β3 subunit is sufficient for the interaction of αvβ3 with the RTKs.

The β3 Extracellular Domain–RTK Interaction Is Functionally Important—We have previously shown that the attachment of cells to a substrate through the αvβ3 integrin enhances the ability of insulin and PDGF to stimulate cell proliferation and migration (9, 11). We used the migration assay to test the ability of the β subunit chimeras to affect PDGF activity. Cells transfected both with β subunit containing the β3 extracellular domain and the PDGF-Rβ responded to PDGF with increased migration, whereas cells with the β1 extracellular domain (β1/3 chimera) did not (Fig. 9). This response was seen both on vitronectin and fibronectin surfaces. Controls indicated that each of the cell lines attached to vitronectin and fibronectin (a prerequisite for migration). The attachment to fibronectin was

![Figure 6](image1.png)

**Fig. 6.** PDGF-Rβ overexpression prevents association of VEGF-R2 with αvβ3. CHO cells were transfected with the β3 subunit and the indicated amount of PDGF-Rβ and VEGF-R2 plasmid DNA. Cell extracts were immunoprecipitated with an antibody against the β3 cytoplasmic tail and immunoblotted with anti-VEGF-R2 (A). Membranes were stripped and reprobed with anti-PDGF-Rβ (B).

Similar outcomes were obtained with PDGF-Rβ3. These results indicate that the cytoplasmic domain of β3 is not needed for the interaction of αvβ3 with VEGF-R2 and PDGF-Rβ3.

![Figure 7](image2.png)

**Fig. 7.** The β3 subunit cytoplasmic tail is not required for the interaction of the integrin with PDGF-Rβ and VEGF-R2. CHO cells were transfected with αv and VEGF-R2 (lanes 1–3) or PDGF-Rβ (lanes 4–6). In addition, the cells were transfected with wild-type β3 (lanes 2 and 5), β3cyto (lanes 3 and 6), or with the control vector pcDNA3 (lanes 1 and 4). Extracts were immunoprecipitated with anti-αvβ3 antibodies and analyzed for the presence of the RTKs by immunoblotting with anti-VEGF-R2 (lanes 1–3) or PY20 (PDGF-Rβ) (lanes 4–6).

![Figure 8](image3.png)

**Fig. 8.** The extracellular domain of the β3 subunit mediates the interaction of β3 with PDGF-Rβ and VEGF-R2. CHO cells were transfected with αv and either VEGF-R2 (lanes 1–4) or PDGF-Rβ (lanes 5–8). In addition, the cells were transfected with wild-type β3 (lanes 1 and 5), the β3/1 chimera (lanes 2 and 6), the β1/3 chimera (lanes 3 and 7), or the wild-type β1 subunit (lanes 4 and 8). Wild-type β3 and the β3/1 chimera were immunoprecipitated with antibodies against the cytoplasmic tail of β3 (lanes 1, 2, 5, and 6), and wild-type β3 and the β1/3 chimera were precipitated with antibodies against the cytoplasmic tail of β3. Immuneoprecipitates were analyzed for the presence of RTKs by immunoblotting with anti-VEGF-R2 (lanes 1–4) or anti-PDGF-Rβ (lanes 5–8).
equal for each of the three cell lines. The $\beta_{1/3}$ cells attached slightly less efficiently to surfaces coated with low concentrations of vitronectin than the other two lines but attached equally well at the concentration used in the migration assay (data not shown). These results show that the biological effects on RTKs correlate with the presence of the $\beta_3$ extracellular domain, the domain that also mediates the physical interaction of the $\alpha_\beta_3$ integrin with RTKs.

**DISCUSSION**

The main new finding in this work is that both PDGF-R$\beta$ and VEGF-R2 associate with the extracellular domain of the $\beta_3$ integrin subunit. Ligand binding and phosphorylation of the RTKs do not seem to be required for the integrin interaction. Our results confirm previous studies that have shown a specific association of PDGF-R$\beta$ (9, 10) and VEGF-R2 (12) with the $\alpha_\beta_3$ integrin. However, our results differ from earlier observations that indicated the RTK-integrin interaction only occurs after cells are stimulated with the relevant growth factor. In contrast, we found the association to be independent of growth factor stimulation and phosphorylation of the RTK. It may be that the expression of the constituent components of the complex was higher than in the earlier work and that this facilitated the detection of the interaction. Stimulation of cells with growth factors induces localization of RTKs to focal contacts (8), thereby increasing the local concentration at sites of integrins. This increased concentration may have favored the formation of the integrin-RTK complexes that were reported in the earlier work.

Our results show that the $\beta_3$ subunit is critical to the RTK association of the $\alpha_\beta_3$ integrin. This result agrees with earlier studies showing that other $\beta$ subunits, which form heterodimers with $\alpha_\v$, such as $\beta_1$ or $\beta_3$, do not associate with PDGF-R$\beta$ or VEGF-R2 (9, 12). A new observation in the present work is that the $\alpha_\v$ subunit is necessary for integrin association of VEGF-R2. Thus, unlike PDGF-R$\beta$, VEGF-R2 was not coprecipitated with the $\alpha_\v\beta_3$ integrin or with $\beta_3$ subunits separated from $\alpha_\v$. This result shows that the two RTKs interact with the $\beta_3$ integrins somewhat differently and that the $\alpha_\v\beta_3$ integrin can affect the cellular response to PDGF-R$\beta$ but not to VEGF-R2. The physiological significance of the interaction between PDGF-R$\beta$ and $\alpha_\v\beta_3$ remains to be determined. The fact that high expression levels of PDGF-R$\beta$ inhibited the association of VEGF-R2 with $\alpha_\v\beta_3$, whereas the reverse was not the case, may also reflect differences in how the two RTKs interact with $\alpha_\v\beta_3$.

A surprising finding is that the integrin-RTK association is determined by the extracellular domain of the $\beta_3$ subunit of the integrin. Growth factor stimulation induces RTK phosphorylation in the intracellular domain. Subsequently, a variety of signaling molecules are recruited to the RTK, resulting in the formation of large intracellular complexes. Integrins also form intracellular complexes, which consist of signaling molecules and cytoskeletal proteins, in response to ligand binding. These complexes accumulate in focal contacts. One might expect that the molecules of these complexes bring together the integrins and the RTKs. However, our integrin truncation and domain-swapping experiments clearly show that the cytoplasmic domain of the $\beta_3$ integrin is not required for association with the RTKs and that the interaction is determined by the extracellular domain of the $\beta_3$ subunit. This conclusion is supported by the finding that phosphorylation of the receptors is not necessary for their association with the $\alpha_\beta_3$ integrin. Furthermore, as truncation of the $\beta_3$ cytoplasmic domain prevents the integrin from accumulating in focal adhesions (19), focal adhesions do not appear to be necessary for the interaction of $\alpha_\beta_3$ with PDGF-R$\beta$ or VEGF-R2. The exact nature of the complex containing $\alpha_\beta_3$ and the RTKs is unclear. Both direct interaction and binding mediated by a third component are viable possibilities. One possible candidate for a third molecule mediator is the integrin-associated protein (IAP-50 or CD47), which is a transmembrane protein that selectively interacts with the extracellular domain of the $\beta_3$ integrin subunit (20).

An alternative way for RTKs to associate with integrins is through focal adhesion kinase, which binds directly to PDGF-R$\beta$ and EGF-R (21). However, as this interaction is not specific for the $\beta_3$ integrin subunit, it is different from the interaction described here and is unlikely to account for the functional cooperation of $\beta_3$ integrins with RTKs.

The association of integrins with RTKs is functionally important. Thus, the ability of the receptors for insulin, PDGF, and VEGF to respond to their growth factor ligands by inducing increased cell proliferation and migration is augmented in the presence of $\alpha_\beta_3$ that has bound to one of its extracellular matrix ligands (9–12). The present results show that the ability of the $\alpha_\beta_3$ integrin to enhance the activity of PDGF-R$\beta$ in cell migration assays is dependent on the $\beta_3$ subunit extracellular domain. This result strongly suggests that the integrin RTK extracellular domain interactions we describe here are the physical basis of the functional cooperativity between $\alpha_\beta_3$ and RTKs. The integrin-RTK cooperation may make it possible for cell attachment to induce an RTK response independently of the growth factor ligand. Cells that attach to fibronectin (a ligand of $\alpha_\beta_3$) show autophosphorylation of PDGF-R$\beta$ (6) and relocation of the receptor to focal contacts (8). Concentration of integrin-associated RTKs to focal adhesions may explain the finding that a highly phosphorylated subfraction of PDGF-R$\beta$ is associated with $\alpha_\v\beta_3$ (9). This subfraction of PDGF-R$\beta$ may also contribute to integrin-mediated signaling, as shown for the...
EGF-receptor and β1 integrins (7). These interactions are likely to be important modulators of growth factor activity in vivo.

Both the αβ3 integrin and two of the associated RTKs, VEGF-R2 and PDGF-Rβ, are important to angiogenesis (22, 23). An understanding of the mechanism underlying the αβ3 integrin-RTK cooperation may lead to the development of useful compounds for modulating the activity of these growth factors. Soldi et al. (12) have shown that anti-αβ3 antibodies can inhibit VEGF-induced VEGF-R2 phosphorylation and cell migration when endothelial cells are bound to the αβ3 ligand vitronectin. As these antibodies do not interfere with cell attachment, they may disrupt the association between the αβ3 integrin and VEGF-R2. Such antibodies, and other compounds capable of interfering with this integrin-RTK interaction, could be valuable in preventing unwanted angiogenesis such as that in tumors, arthritic synovium, and the retina.

Acknowledgments—We thank Dr. Carl-Henrik Heldin for PDGF-Rβ cDNA, Dr. Georg Breier for the VEGF-R2 cDNA, and Dr. Mark Ginsberg for the αIIbβ3-expressing CHO cells.

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Platelet-derived Growth Factor Receptor \( \beta \) and Vascular Endothelial Growth Factor Receptor 2 Bind to the \( \beta_3 \)Integrin through Its Extracellular Domain
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*J. Biol. Chem.* 2000, 275:39867-39873.  
doi: 10.1074/jbc.M007040200 originally published online August 29, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M007040200

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