A Biochemical Characterization of the Binding of Osteopontin to Integrins $\alpha_v\beta_1$ and $\alpha_v\beta_3$*

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Osteopontin (OPN) is an extracellular matrix protein that binds to integrin $\alpha_v\beta_3$. Here we demonstrate that two other integrins, $\alpha_v\beta_1$ and $\alpha_v\beta_5$, are also receptors for OPN. Human embryonic kidney 293 cells adhere to human recombinant osteopontin (glutathione S-transferase-osteopontin; GST-OPN) using integrin $\alpha_v\beta_1$. When the 293 cells are transfected with the $\beta_3$ subunit, they can also adhere to GST-OPN using integrin $\alpha_v\beta_3$. Divalent cations regulate the binding of GST-OPN to both $\alpha_v\beta_1$ and $\alpha_v\beta_3$. Mg$^{2+}$ and Mn$^{2+}$ support the binding of GST-OPN to these integrins but Ca$^{2+}$ does not. The highest affinity is observed in Mn$^{2+}$. In the presence of this ion, the affinity for GST-OPN to $\alpha_v\beta_1$ is 18 nM and the affinity for $\alpha_v\beta_3$ is 48 nM. The antibody 8A2, which is an agonist for $\alpha_v$, promotes the adhesion of 293 cells to GST-OPN even when Ca$^{2+}$ is present. This observation suggests that cellular events could modulate the affinity of $\alpha_v\beta_3$ for OPN. Collectively, these findings prove that integrins $\alpha_v\beta_1$, $\alpha_v\beta_2$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$ have similar affinity for OPN. Therefore, all three integrins must be considered when evaluating the biological effects of OPN.

Osteopontin (OPN) is a secreted phosphoprotein that was originally isolated from bone (1). OPN is also found in many other fluids and tissues including milk, urine, placenta, kidney, leukocytes, smooth muscle cells, and some tumor cells (for reviews, see Refs. 1 and 2). OPN supports cell adhesion through its Arg-Gly-Asp (RGD) integrin recognition motif. OPN is also rich in aspartic acid residues, and can be heavily glycosylated. The acidic nature of OPN probably accounts for its ability to modulate the growth of calcium crystals in both bone (1, 2) and urine (3).

Integrin $\alpha_v\beta_3$ is the established receptor for OPN. In bone, $\alpha_v\beta_3$ is expressed on osteoclasts and it initiates bone resorption by mediating adhesion of the osteoclast to OPN in bone (4–6). It has also been hypothesized that OPN and integrin $\alpha_v\beta_3$ facilitate vascular remodeling because these two proteins are co-localized in smooth muscle cells following balloon angioplasty (7). Both OPN and integrin $\alpha_v\beta_3$ are also present in human placenta (8, 9), so their interaction could also be relevant to pregnancy.

Although $\alpha_v\beta_3$ is clearly a receptor for OPN, many other integrins also bind the RGD motif (10, 11) and no data have excluded other integrins as receptors for OPN. Therefore, we hypothesized that other integrins with the $\alpha_v$ subunit may also bind OPN. The purpose of this study was to provide a quantitative biochemical analysis of the binding of OPN and integrins $\alpha_v\beta_1$ and $\alpha_v\beta_5$. We reason that a measure of these binding affinities will allow a meaningful comparison with the binding affinity of OPN to $\alpha_v\beta_3$ (12). If more than one integrin does bind OPN with similar affinity, then much information attributing adhesion and signaling events entirely to the interaction between OPN and $\alpha_v\beta_3$ should be re-evaluated.

**MATERIALS AND METHODS**

Cell Lines—Human embryonic kidney carcinoma 293 cells were obtained from ATCC and maintained in Dulbecco's modified Eagle's medium (Bio Whittaker) supplemented with 10% fetal calf serum (Irvin Scientific), 20 mM Hepes (pH 7.4), 1% glutamine, 1% penicillin, and 1% streptomycin (Sigma). Human embryonic kidney (HEK) 293 cells were transfected at passage 40 with $\mu$g pcDNA3 or pcDNA3 vector alone using N-(1-ethyl-3-3-dimethylammoniumpropane)sulfate (N,N,N,N-tetramethylammonium methylsulfate transfection reagent (Boehringer Mannheim). Stable transfectants were obtained after selection in 500 gM G418 (G418) for 2 weeks and maintained thereafter in 250 gM G418. Cells expressing high levels of $\alpha_v\beta_3$ were obtained by sterile FACS with an anti-$\beta_3$ monoclonal antibody (mAb), P3G2.

**Protein Expression and Purification**—In this study a recombinant form of OPN fused the glutathione S-transferase (GST-OPN) was used as ligand. We have previously described the characterization of this ligand (12). GST-OPN supports cell adhesion in a manner equivalent to native uropontin, a form of OPN purified from human urine (12). We have also found that both versions of OPN function equally in supporting cell adhesion through integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (data not shown). GST-OPN was chosen in the interest of consistency in performing cell binding studies and because of its availability. Integrin $\alpha_v\beta_3$ was purified from a human placental extract using monoclonal antibody affinity chromatography as described previously (13). The identity and purity of this protein was assessed by N-terminal amino acid sequencing and by its ability to bind a series of monoclonal antibodies specific for either $\alpha_v\beta_3$ or $\alpha_v\beta_5$.

Vitronectin was purified from human plasma by affinity chromatography on heparin-Sepharose as described (14).

**Antibodies**—The monoclonal antibody 8A2 and its Fab fragment bind to the integrin $\beta_3$ subunit and stimulate the ligand binding function of integrins containing this subunit. An in-depth characterization of this antibody has been published (15, 16). Monoclonal antibody L230 (anti-$\alpha_v$) was purified from cell culture supernatant from hybridoma cells (ATCC, HB8448) by using protein A-Sepharose. The blocking activity of this antibody has been reported previously (17). Monoclonal antibody P4C10 (anti-$\beta_3$) was purchased from Life Technologies, Inc. and was used in ascertaining, normally at a dilution of 1:500. Anti-$\beta_3$, monoclonal

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§§The abbreviations used are: OPN, osteopontin; GST-OPN, recombinant osteopontin that is a fusion protein with glutathione S-transferase; RGD, Arg-Gly-Asp; mAb, monoclonal antibody; FACS, fluorescence-activated cell sorting.
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**Fig. 1.** FACS analysis of integrin expression on kidney 293 cells. A panel of monoclonal antibodies was used to assess integrin expression on wild-type and \(\beta_5\)-transfected human kidney 293 cells. Cells were incubated with mouse IgG or with the primary antibodies and then with secondary fluorochrome-conjugated goat anti-mouse IgG. Following extensive washing to remove free antibody the cells were analyzed by flow cytometry. The expression level of each integrin subunit is indicated by the mean fluorescence intensity. The integrin expression profile of wild-type 293 cells was compared with mAb LM609 against \(\alpha_5\beta_1\) (A), P3G2 against \(\alpha_v\beta_5\) (B), 14H4 against \(\alpha_v\) (C), and mAb 1977 against \(\beta_5\) (D). Following transfection of these cells with the cDNA for \(\beta_5\), the expression of the \(\alpha_v\beta_5\) heterodimer was detected with mAb P3G2 (E). Cells transfected with the vector pcDNA3 alone exhibited a profile identical to wild-type 293 cells (not shown).
Osteopontin Binds to Integrins \( \alpha_v \beta_1 \) and \( \alpha_v \beta_5 \)

**Fig. 2.** Wild-type 293 cells and \( \beta_5 \)-transfected 293 cells adhere to OPN. The adhesion of wild-type (open bars) and \( \beta_5 \)-transfected (dark bars) 293 cells to GST-OPN was challenged with a series of blocking monoclonal antibodies. Cell adhesion to GST-OPN was performed in the presence of LM609 (anti-\( \alpha_v \beta_1 \)), P1F6 (anti-\( \alpha_v \beta_5 \)), and L230 (anti-\( \alpha_v \)).

![Image](image_url)

**Fig. 3.** A comparison of the effects of divalent ions on cell adhesion to osteopontin and vitronectin. The adhesion of kidney 293 cells expressing either integrin \( \alpha_v \beta_1 \) (panels A and C) or integrin \( \alpha_v \beta_5 \) (panels B and D) to either GST-OPN (panels A and B) or vitronectin (panels C and D) was tested in buffer containing \( \text{Ca}^{2+} \), \( \text{Mg}^{2+} \), or \( \text{Mn}^{2+} \). The adhesion of the \( \beta_5 \)-transfected cells was measured in the presence of antibody P4C10 to eliminate any contribution of endogenous \( \alpha_v \beta_1 \) to cell adhesion. Adhesion assays were conducted as described under “Experimental Procedures.” Each data point is the average of quadruplicate measurements. This experiment was performed four times yielding identical results. Additionally, in separate experiments, identical results were obtained when uropontin was used as immobilized ligand.

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\( \alpha_v \beta_5 \) on 293 cells was also confirmed by immunoprecipitation and Western blotting using antibodies specific for \( \alpha_v \beta_5 \) (data not shown).

Integrins \( \alpha_v \beta_1 \) and \( \alpha_v \beta_5 \) are Receptors for OPN—To determine whether \( \alpha_v \beta_1 \) and \( \alpha_v \beta_5 \) could mediate cell adhesion to GST-OPN, the wild-type and \( \beta_5 \)-transfected 293 cells were allowed to adhere to immobilized GST-OPN. Both cell lines adhere to GST-OPN (Fig. 2). The adhesion was blocked by RGD peptide and by P4C10, an antibody against the \( \beta_5 \) subunit. The adhesion of these cells was also inhibited by L230, an antibody that blocks function of \( \alpha_v \). The antibody against \( \alpha_v \beta_5 \) LM609, had no effect. Based on these data, and immunoprecipitation experiments showing that the majority of \( \beta_5 \) in these cells is complexed with \( \alpha_v \) (data not shown), we conclude \( \alpha_v \beta_5 \) is a receptor for OPN.

The adhesion of \( \beta_5 \)-transfected 293 cells was also blocked by the antibody against the \( \alpha_v \) subunit (L230). Approximately 70% of the adhesion of the \( \beta_5 \)-transfected cells could be blocked by P1F6, an antibody that interferes with ligand binding to \( \alpha_v \beta_5 \). The remainder of the adhesion (30%) could be blocked by antibody against the \( \beta_5 \) subunit, indicating that the endogenous \( \alpha_v \beta_5 \) contributes to the adhesion of these cells to OPN. These experiments show that \( \alpha_v \beta_5 \) can also mediate cell adhesion to OPN.

**Fig. 3**

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The Cation Dependence of Adhesion to OPN Is Distinct from the Cation Dependence for Vitronectin—\( \text{Ca}^{2+} \) does not support the binding of OPN to \( \alpha_v \beta_1 \) (12). To determine if \( \text{Ca}^{2+} \) is similarly ineffective in supporting GST-OPN binding to \( \alpha_v \beta_1 \) and \( \alpha_v \beta_5 \), we tested the ability of \( \text{Ca}^{2+} \), \( \text{Mg}^{2+} \), and \( \text{Mn}^{2+} \) to support the adhesion of wild-type and \( \beta_5 \)-transfected 293 cells to GST-OPN (Fig. 3, panels A and B). For comparison, the ability of each ion to support the adhesion of each cell line to vitronectin is also shown (panels C and D). In this study, the amount of coated protein was varied across a concentration range. Each ion was used at a concentration found to support maximal adhesion (not shown). \( \text{Ca}^{2+} \) did not support adhesion of either cell line to GST-OPN. However, \( \text{Ca}^{2+} \) did enable maximal cell adhesion to vitronectin. \( \text{Mn}^{2+} \) was most effective in supporting the adhesion of \( \alpha_v \beta_1 \) and \( \alpha_v \beta_5 \) expressing cells to GST-OPN. \( \text{Mg}^{2+} \), which is likely to be the physiologically relevant ion, also supported adhesion. Despite slight differences in the rank order potency of divalent ions in supporting adhesion to vitronectin, all three ions did support maximal adhesion to this protein. Physiologic levels of \( \text{Ca}^{2+} \) supported adhesion to vitronectin but not to GST-OPN. We conclude that there is a fundamental difference in the cation requirement of integrin binding to OPN as opposed to vitronectin.

Measuring the Affinity of GST-OPN for \( \alpha_v \beta_1 \) and \( \alpha_v \beta_5 \)—The OPN receptor that has received the most attention is integrin \( \alpha_v \beta_1 \). We recently measured the affinity between GST-OPN and purified integrin \( \alpha_v \beta_1 \) and found the apparent \( K_d \) to be between 5 and 30 nM (12). Recent binding studies between GST-OPN and \( \alpha_v \beta_3 \) on the surface of M21 melanoma cells has yielded a similar affinity (data not shown). To gauge the significance of the binding of OPN to \( \alpha_v \beta_1 \) or \( \alpha_v \beta_5 \), it is important to compare the binding affinities between OPN and each of these integrins. To measure the affinity of OPN for \( \alpha_v \beta_1 \) and \( \alpha_v \beta_5 \), we performed binding assays with soluble \( ^{125} \text{I}- \)GST-OPN. These binding studies were performed by harvesting the wild-type 293 cells or the \( \beta_5 \)-transfected 293 cells from tissue culture flasks and placing the cells in suspension. Binding studies were done in \( \text{Mn}^{2+} \) to obtain the highest affinity between GST-OPN and the two integrins. In the case of the \( \beta_5 \)-transfected cells, we found that a small component (typically 10–20% of total binding) of GST-OPN binding was mediated through endogenous \( \alpha_v \beta_5 \). To eliminate this component from the analysis, the binding studies with the \( \beta_5 \)-transfected cells were performed in the presence of a saturating level of a function blocking antibody against the \( \beta_5 \) subunit. Initial control binding studies showed that the specific binding of \( ^{125} \text{I}- \)GST-OPN to both wild-type and \( \beta_5 \)-transfected 293 was inhibited completely by an RGD peptide and by blocking antibody against the \( \alpha_v \) subunit (data not shown). To measure the relative affinity of GST-OPN for integrin \( \alpha_v \beta_1 \) and \( \alpha_v \beta_5 \), binding isotherms were generated across a concentration range of \( ^{125} \text{I}- \)GST-OPN (Fig. 4). Scatchard analysis of the binding isotherms revealed that OPN has an affinity of 18 nM for \( \alpha_v \beta_1 \) (Fig. 4B) and 48 nM for \( \alpha_v \beta_5 \) (Fig. 4D). These affinity constants are similar to the apparent \( K_d \) (5–30 nM) we measured between GST-OPN and purified \( \alpha_v \beta_1 \) (12). Consequently, the binding affinity between GST-OPN and all three \( \alpha_v \)-integrins is similar.

Binding of GST-OPN to Purified Integrin \( \alpha_v \beta_5 \)—Integrin
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$\alpha_v\beta_5$ is abundant enough in placenta to purify $\alpha_v\beta_5$ for direct binding studies (13). We measured the binding of 125I-GST-OPN to purified $\alpha_v\beta_5$ using the same format that was previously used for $\alpha_v\beta_3$ (12). As shown in Fig. 5A, $\text{Mn}^{2+}$ is more effective than $\text{Ca}^{2+}$ in promoting the binding of OPN to $\alpha_v\beta_5$. Although this assay format does not allow an exact derivation of $K_d$, because the binding of ligand to integrin is irreversible in this assay format (11), we can assign an apparent $K_d$ and compare this value to that obtained for $\alpha_v\beta_3$. In $\text{Mn}^{2+}$, the apparent $K_d$ of GST-OPN for $\alpha_v\beta_5$ is 20 nM, which is comparable to the value of 5–30 nM for $\alpha_v\beta_3$ (12). Thus, the two purified integrins bind GST-OPN with nearly equal affinity. The purified $\alpha_v\beta_3$ is obtained from a placental lysate by first depleting the lysate of $\alpha_v\beta_3$ by affinity chromatography. Therefore, we performed an enzyme-linked immunosorbent assay on the purified $\alpha_v\beta_3$ to make sure that it contained no contaminating $\alpha_v\beta_5$. This enzyme-linked immunosorbent assay was done with mAb 6B9 which is specific for $\alpha_v\beta_5$ and mAb LM609 which binds only to $\alpha_v\beta_3$. As shown in Fig. 5B, the purified $\alpha_v\beta_3$ contains no detectable $\alpha_v\beta_5$, proving that OPN binds to purified $\alpha_v\beta_5$.

Adhesion to OPN through Integrin $\alpha_v\beta_3$ Can Be Stimulated by Activation of the $\beta_1$ Subunit with Monoclonal Antibody BA2—It has been reported that many integrins can exist in multiple affinity states (16, 24–30). These observations indicate that there may be cellular pathways that control the affinity of an integrin for its ligand. Because our data shows that $\text{Ca}^{2+}$ does not support adhesion to OPN, we wondered if other stimuli could override this phenomena. Since the physiologic stimuli that regulate integrin affinity have not been completely discerned, we made use of the monodonal antibody BA2. This antibody is a known agonist for $\beta_1$ integrins (15, 16) and it has been suggested that BA2 mimics the physiologic activation of these integrins. We tested the ability of BA2 to stimulate the adhesion of 293 cells to OPN. These studies were performed in buffer containing $\text{Ca}^{2+}$. As shown in Fig. 6A, 293 cells adhered to GST-OPN in the presence of mAb BA2 in buffer containing $\text{Ca}^{2+}$. No adhesion to GST-OPN was observed in the presence of normal mouse IgG in the same buffer. To determine whether this stimulation was saturable and corresponded with the binding of BA2 to $\alpha_v\beta_3$, the number of cell surface binding sites for the antibody was measured. As shown in Fig. 6B the binding of 125I-mAb BA2 to 293 cells in suspension approaches saturation between 0.5 and 1 $\mu$g/ml of antibody. This concentration corresponds closely with the amount of the antibody that maximally stimulates adhesion to OPN (Fig. 6A). From the Scatchard plot shown in Fig. 6C, the $K_d$ of mAb 8A2 for $\alpha_v\beta_1$...
Many interactions between cells and the extracellular matrix depend on cellular recognition of the RGD motif within adhesive proteins. Small peptides with the RGD sequence will bind to several integrin adhesion receptors, but larger adhesive proteins display considerable integrin binding specificity. Therefore, an important issue with every RGD-containing adhesive protein is to identify its receptor(s). OPN, for instance, binds to integrin \( \alpha_v\beta_3 \), but not to the platelet integrin \( \alpha_{IIb}\beta_3 \) (12). However, it is now apparent that several integrins have ligand binding properties similar to \( \alpha_v\beta_3 \). These are the four other integrins containing the \( \alpha_v \) subunit, \( \alpha_5\beta_1, \alpha_5\beta_3, \alpha_6\beta_1, \) and \( \alpha_7\beta_1 \) (22). Like \( \alpha_v\beta_3 \), two of these integrins, \( \alpha_5\beta_1 \) and \( \alpha_5\beta_3 \), bind to vitronectin. This functional similarity leads us to suspect that both of these integrins may also bind OPN. Since both \( \alpha_v\beta_1 \) and \( \alpha_v\beta_3 \) have been identified in tissues, like bone and the vasculature where OPN is involved in tissue remodeling (1, 2, 31), there is the potential for a physiologically relevant interaction between these integrins and OPN.

Ideally experiments designed to characterize the interactions between integrins and their ligands would provide a quantitative measure of these interactions so that a hierarchy of binding affinities is available. Here, the affinity between OPN and integrin \( \alpha_v\beta_1 \) and \( \alpha_v\beta_3 \) was determined by measuring the binding of \( ^{125}I \)-GST-OPN to these integrins present on the surface of kidney 293 cells. Scatchard analysis shows that in the presence of \( \alpha_v\beta_1 \), the affinity and number of binding sites on 293 cells for mAb 8A2 was measured by number of binding sites on 293 cells for mAb 8A2 was measured by

DISCUSSION

Many interactions between cells and the extracellular matrix depend on cellular recognition of the RGD motif within...
ably, the apparent $K_d$ (20 nm) between GST-OPN and purified integrin $\alpha_v\beta_3$ is comparable to the affinity between GST-OPN and purified $\alpha_\text{v} \beta_3$ measured in the same assay under the same conditions (12). In addition, several cell adhesion experiments showed that the coating concentration of GST-OPN necessary for half-maximal cell adhesion through $\alpha_\text{v}\beta_3$ is comparable to the affinity between GST-OPN and vitronectin to $\alpha_v\beta_3$. This observation is important because it illustrates a key difference between the binding of OPN and vitronectin to $\alpha_v\beta_3$. Although small differences exist in the rank-order potency of divalent ions in supporting adhesion to vitronectin, physiologic levels of Ca$^{2+}$ supported maximal cell adhesion to this protein through $\alpha_\text{v}\beta_3$, and $\alpha_\text{v}\beta_3$. This in contrast to the adhesion to OPN which is not supported at any level by Ca$^{2+}$. In this regard it is worth noting an important biochemical distinction between vitronectin and OPN. The vitronectin used in these studies is a multimer, often containing between 12 and 15 vitronectin moieties per multimer (32, 33). There is substantial evidence that the multimeric vitronectin is also present in extracellular matrices in vivo (32–34). In contrast, the OPN used in these studies was proven to be monomeric by mass spectral analysis (12) and gel filtration chromatography (data not shown). The soluble OPN found in body fluids is also assumed to be a monomer. Consequently, it is possible that multimeric vitronectin engages several integrins simultaneously, thereby overriding an otherwise lower affinity between vitronectin and $\alpha_v\beta_3$ integrins in calcium ion. While Ca$^{2+}$ does not support OPN binding to integrins $\alpha_v\beta_1$ and $\alpha_v\beta_3$, Mg$^{2+}$ is able to enhance the binding. This result is not unexpected because Mg$^{2+}$ is known to activate ligand binding functions of many integrins (22, 35–38). The physiologic activation of integrins can also be mimicked by mononodal antibodies (16, 39–41). For example, several studies have demonstrated that integrins can be subject to physiologic activation. The best example is the platelet fibrinogen receptor integrin $\alpha_{\text{IIb}}\beta_3$ which exists in a dormant state on resting platelets. This integrin responds to platelet activation by increasing its affinity for soluble fibrinogen (42). This increased binding affinity enables platelet aggregation at the site of a wound. Our data indicate that the binding of GST-OPN to integrin $\alpha_\text{v}\beta_3$ can be enhanced by both Mg$^{2+}$ and the mAb BA2, which is known to be an agonist of other $\beta_3$-integrins. Although several other integrins are known to have agonists other than divalent ions (16), to our knowledge, this is the first demonstration that the affinity of an $\alpha_v$-integrin can be modulated by an agonist besides Mg$^{2+}$. By analogy with other integrins that are similarly stimulated, it is possible that this artificial stimulus indicates the potential for enhancing the affinity state of the integrin by physiologic means. It is important to emphasize that even when Ca$^{2+}$ is present, the mAb BA2 was able to enhance cell adhesion to OPN to maximal levels. Thus, the suppressive effects of Ca$^{2+}$ can be overridden by other stimuli. In future studies, it will be important to determine if $\alpha_\text{v}\beta_3$ and $\alpha_v\beta_3$ can be similarly stimulated to bind OPN when Ca$^{2+}$ is present and to determine if there are cellular signals that can promote adhesion to OPN in Ca$^{2+}$.

The binding of OPN to $\alpha_v\beta_1$ and $\alpha_v\beta_3$ may be important to bone homeostasis. OPN is thought to be one of the most important matrix proteins for osteoclast adhesion (2, 4). In addition, soluble OPN stimulates intracellular signaling in osteoclasts, including Ca$^{2+}$ fluxes and the phosphorylation of intracellular proteins (43). It has been reported that integrin $\alpha_\text{v}\beta_3$ is present on human osteoclasts (44–47) and that integrin $\alpha_\text{v}\beta_1$ is present on chicken osteoclast precursors (48, 49). Therefore both of these integrins are positioned to mediate interactions between OPN and cells in bone. Our findings that integrins $\alpha_v\beta_1$ and $\alpha_v\beta_3$ have high affinity for OPN indicates that interactions between OPN and these receptors may play an essential role in bone remodeling. Blocking the activity of $\alpha_v\beta_3$ with antibodies inhibits its bone resorption, but no analogous study has been done with antagonists of other $\alpha_v$-integrins. Our data suggest that similar experiments should be done with antagonists of $\beta_1$ and $\beta_3$.

Recent study also indicates that OPN is involved in vascular injury and repair (6, 31). One of the initial responses to vascular injury is the formation of a neointima which precedes the formation of atherosclerotic lesions (50). Giachelli et al. (51) recently showed that OPN expression is increased substantially in both rat and human smooth muscle cells surrounding a vessel that has been exposed to a catheter-injured vessel. Because of the temporal regulation of OPN synthesis following this insult, the hypothesis was put forth that the OPN expressed by smooth muscle cells may be an important modulator of cell migration and proliferation associated with neointima formation (7, 52). The same group showed that, integrin $\alpha_v\beta_3$ mediates only a portion of smooth muscle cell or to OPN; a major component of this adhesion was not blocked by antagonists specific for $\alpha_v\beta_3$. The data presented in this report indicate that integrins $\alpha_v\beta_3$ and $\alpha_v\beta_1$ should be considered as candidate OPN receptors involved in guiding vascular repair.

The kinetic data in this report provide information essential to an understanding of the biology of OPN. Many adhesive and signaling events are tied to cellular exposure to OPN. In large part, it had been assumed that these events are mediated by integrin $\alpha_v\beta_3$ because it was the only known OPN receptor. In conjunction with our prior study (12), the data in this report show that $\alpha_v\beta_3$ and $\alpha_v\beta_1$ have similar affinities for OPN and that the ion regulation of OPN binding to each integrin is nearly identical. Therefore, along with $\alpha_v\beta_1$ and $\alpha_v\beta_3$ $\alpha_v\beta_3$ must now be considered receptors for OPN.

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