inhibitors to discrepancy between our results and those obtained using (Table 1). A number of factors may account for the apparent hyperplasia after vascular injury in other animal models may influence the contribution of

...to develop intimal hyperplasia. Our findings in the β₃−/− mice stand in stark contrast to a large body of data indicating that antagonists of integrin αᵥβ₃ (and, in some cases, α₃β₁) reduce intimal hyperplasia after vascular injury in other animal models (Table 1). A number of factors may account for the apparent discrepancy between our results and those obtained using inhibitors to αᵥβ₃ (and, in some cases, α₃β₁). First, differences in animal species and/or technical features of the models may influence the contribution of β₃-integrins to intimal hyperplasia. Previous models have involved balloon catheter injury induced in rabbits, rats, and guinea pigs; catheter injury in hamsters; stent implantation in pigs, monkeys, and baboons; and arterial cuff injury in rabbits (Table 1). In contrast to these models, our model utilizes mice and involves a combination of guidewire-induced endothelial denudation and arterial ligation; the latter maneuver, which is known to enhance or produce intimal hyperplasia in other models, was not used in the previous studies using antagonist to αᵥβ₃. Nonetheless, the protection from intimal hyperplasia we observed with the P-selectin−/− mice is very similar to that obtained both in a mouse model utilizing arterial ligation to initiate vascular injury and in a rat model involving balloon catheter injury. Second, loss of α₃β₁ on a genetic basis may result in compensatory increases in the number and/or affinity of other adhesion receptors, whereas such compensation probably cannot occur with acute inhibition of αᵥβ₃. For example, we previously demonstrated that Glanzmann thrombasthenia patients with abnormalities in α₃β₁ had increased numbers of platelet αᵥβ₃ receptors. In addition, in contrast to the effect of inhibiting an integrin receptor, the absence of the receptor may affect signaling mediated by other integrins by virtue of decreased binding of intracellular proteins involved in signaling that ordinarily bind to the cytoplasmic domain of the missing integrin. Finally, as opposed to inhibiting the α₃β₁ receptor, its absence in β₃−/− mice may result in the loss of transduction of signals initiated by αᵥβ₃; such signals may be initiated by the unliganded receptor, the liganded receptor, or the antagonized receptor.

In sharp contrast to the failure of β₃-integrin deficiency to protect against the development of intimal hyperplasia after vascular injury, P-selectin deficiency offered dramatic protection. Because this protection correlated with nearly complete absence of leukocyte recruitment to the platelets lining the vessel 1 hour after injury, it is possible that leukocyte recruitment is a crucial element in the development of intimal hyperplasia. However, the platelets that deposited on the damaged blood vessel wall of the P-selectin−/− mice were less compact and retained more of their granular contents than the platelets that deposited on the blood vessel surface of wild-type mice, which suggests that the platelets may be less activated. Our observations on platelet thrombus formation in P-selectin−/− mice are consistent with several other observations that indicate that P-selectin plays an important role in platelet function. Thus, P-selectin−/− mice have prolonged

### Table 2. Platelet Deposition, Platelet Granule Number, and Height of Platelet Thrombi Deposited on Denuded Endothelial Surface in C57Bl/6 Wild-Type and P-Selectin−/− Mice 1 Hour After Injury

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Granules/μm²</th>
<th>Granules/plt</th>
<th>Plt/μm²</th>
<th>Height, μm†</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57Bl/6 WT</td>
<td>6.6 ± 0.7</td>
<td>2.3 ± 0.2</td>
<td>2.9 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>P-selectin−/−</td>
<td>15.9 ± 1.9</td>
<td>3.1 ± 0.2</td>
<td>5.2 ± 0.5</td>
<td>6.6 ± 0.3</td>
</tr>
</tbody>
</table>

P<0.001 P<0.001 P<0.001 P<0.001

†Height (μm) of the platelet layer (mean±SEM) above IEL.

*Mean±SEM expressed per micrometer of IEL.

**Plt indicates platelet.
bleeding times, and P-selectin has been implicated in contributing to both platelet-platelet interactions in vitro and fibrin thrombus formation in vivo. Moreover, Ruggeri et al. presented evidence that platelet thrombi formed from P-selectin−/− mice on collagen-coated surfaces ex vivo under shear are taller and thinner than thrombi formed from wild-type mice. These results are very similar to our in vivo data. Recently, P-selectin glycoprotein ligand-1 (PSGL-1) was detected on platelets, and the platelet GP Ib/IX/V complex has been identified as a counterreceptor for endothelial P-selectin, which raises the possibility that activated platelets expressing P-selectin can interact with activated and unactivated platelets via GP Ib/IX/V and/or PSGL-1. Such interactions may contribute to platelet accumulation, platelet activation, and platelet thrombus formation. Thus, it remains possible that at least some of the protection from the development of intimal hyperplasia in P-selectin−/− mice reflects abnormalities in platelet function rather than abnormal leukocyte recruitment. Nonetheless, a correlation between early leukocyte recruitment and subsequent development of intimal hyperplasia has previously been observed by several investigators using other species, and studies by Simon et al. recently demonstrated that αιβδ-deficient mice (Mac1−/−) were protected from developing intimal hyperplasia after vascular injury. There are a number of plausible links between leukocyte recruitment and subsequent development of intimal hyperplasia, including direct involvement of macrophages and activation of smooth muscle cells by leukocyte elastase, but causality between these phenomena and the mechanisms responsible remain to be established.

Immunohistochemical analysis revealed that platelets were deposited along the vessels in both wild-type and β3-integrin-deficient mice, and TEM demonstrated a single layer of platelets in the β3−/− mice, which indicates that platelet adhesion but not platelet-platelet interactions occur in the absence of αιβδ, presumably via receptors such as GP Ib. β3−/− mice recruited leukocytes to the platelets adherent to the site of vascular injury at 1 hour, but P-selectin−/− mice did not. Several receptor pairs have been implicated in platelet-leukocyte interactions: P-selectin on the surface of

### Table 3. Intimal Hyperplasia After Vascular Injury in Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Intima, μm²</th>
<th>Media, μm²</th>
<th>I/M Ratio</th>
<th>Lumen, μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. WT C57Bl/129</td>
<td>16</td>
<td>14 600±2800</td>
<td>9400±600</td>
<td>1.45±0.2</td>
<td>21 000±3100</td>
</tr>
<tr>
<td>β3−/− C57Bl/129</td>
<td>16</td>
<td>12 700±1000</td>
<td>9000±800</td>
<td>1.3±0.1</td>
<td>24 600±2700</td>
</tr>
<tr>
<td>B. WT C57Bl/6</td>
<td>16</td>
<td>10 200±2100</td>
<td>10 000±600</td>
<td>1.1±0.3</td>
<td>24 900±3500</td>
</tr>
<tr>
<td>P-Sel−/− C57Bl/6</td>
<td>16</td>
<td>2700±900</td>
<td>8500±600</td>
<td>0.3±0.1</td>
<td>34 100±2200</td>
</tr>
<tr>
<td>C. WT 129Sv</td>
<td>10</td>
<td>20 400±2000</td>
<td>11 300±900</td>
<td>1.9±0.3</td>
<td>23 000±2700</td>
</tr>
</tbody>
</table>
activated platelets and its leukocyte counterreceptor PSGL-1; platelet GP Ib and leukocyte \( \alpha_{M} \beta_{2} \); platelet intercellular adhesion molecule-2 and leukocyte \( \alpha_{4} \beta_{1} \); platelet GP Ib and leukocyte \( \alpha_{M} \beta_{2} \); platelet intercellular adhesion molecule-2 and leukocyte \( \alpha_{4} \beta_{2} \); platelet \( \alpha_{IIb} \beta_{3} \) and/or \( \alpha_{V} \beta_{3} \) bridged by fibrinogen to leukocyte \( \alpha_{M} \beta_{2} \); and platelet and leukocyte CD36 (GP IV) bridged by thrombospondin-1. Our results indicate that neither \( \alpha_{M} \beta_{2} \) nor \( \alpha_{V} \beta_{3} \) is necessary for murine platelet-leukocyte interactions but that P-selectin expression is required. In unpublished studies, we have confirmed that as in humans, murine platelet P-selectin plays a major role in platelet-leukocyte interactions, because antibodies to P-selectin inhibit the binding of murine neutrophils to thrombin-activated murine platelets in vitro (V. Evangelista, MD, S.S. Smyth, MD, PhD, and B.S. Coller, MD, unpublished data, 1999).

In summary, the present study provides evidence that \( \beta_{3} \)-integrin deficiency results in decreased platelet deposition but no protection from intimal hyperplasia, whereas P-selectin deficiency protects against the development of intimal hyperplasia. Because antagonists of \( \alpha_{M} \beta_{2} \) have demonstrated efficacy in preventing acute ischemic complications of percutaneous coronary interventions,\(^{35}\) our data are of potential therapeutic importance, raising the possibility that the addition of an antagonist to P-selectin may provide additional protection against intimal hyperplasia and clinical restenosis.

**Note Added in Proof**

After acceptance of this manuscript, Chico et al (Circulation. 2001;103:1135–1141) reported that antagonists to \( \alpha_{M} \beta_{2} \), \( \alpha_{4} \beta_{1} \), or both \( \alpha_{M} \beta_{2} \) and \( \alpha_{4} \beta_{1} \) prevented neointima formation after porcine coronary artery angioplasty when administered for 14 days.

**Acknowledgment**

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**References**


β3-Integrin–Deficient Mice but Not P-Selectin–Deficient Mice Develop Intimal Hyperplasia After Vascular Injury: Correlation With Leukocyte Recruitment to Adherent Platelets 1 Hour After Injury

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