Two articles in this issue focus attention on the α2β1 integrin, a platelet surface collagen receptor. The articles address the potential contributions of varying α2β1 integrin expression to the manifestation of hemorrhagic or thrombotic disease. For some years, fibrillar collagens have been recognized as the most thrombogenic macromolecular constituents of the vessel wall. Unlike many other components of the extracellular matrix, the collagens not only support platelet adhesion, but also promote platelet activation and subsequent aggregation. Remarkable progress has been made over the last 10 to 12 years in placing the platelet-collagen interaction on a firm mechanistic foundation. Of course, there are still important questions to be answered and details to be filled in, but a reasonable vision of the overall process now seems in hand. Formation of a stable platelet aggregate on collagenous substrates is the end result of a complex series of distinct, but integrated and inter-related, receptor-ligand adhesive interactions.

The critical role of von Willebrand factor (vWF) in mediating platelet deposition onto injured vessel walls in the presence of the shear forces encountered at the interface between flowing blood and the vessel wall has been long appreciated. The platelet adhesion promoting activity of the multimeric vWF is largely mediated by the bridging of vessel wall constituents, such as collagens to which vWF binds, and the glycoprotein Ib-IX-V complex on the platelet surface by vWF. Collagen VI seems to play an especially important role as a vessel wall ligand for vWF. Quantitative deficiencies or qualitative defects in vWF that compromise the platelet adhesion promoting activity give rise to the bleeding disorder, von Willebrand disease.

Recent studies have shed additional insight into the mechanism by which vWF confers shear resistance on adherent platelets and facilitates subsequent downstream hemostatic events. vWF mediates an initial tethering of platelets to the adhesive substrate. Under the influence of shear, platelets translocate along the surface in the direction of flow tethered by vWF until movement is arrested by firm engagement of a second receptor. In the case of collagen-rich substrates, it is the platelet surface α2β1 integrin that engages collagens and halts translocation. This paradigm mechanistically resembles the previously established mechanism of leukocyte adhesion to vessel walls in which tethering and rolling occur via selectin-mediated adhesive interactions with firm attachment and stable adhesion resulting from the subsequent engagement of leukocyte surface β2 integrins.

The α2β1 integrin (glycoprotein Ia-IIa, VLA-2, CD49b/CD29) is the most intensively studied and best understood of the collagen receptors. The α2β1 integrin functions as a collagen receptor not only on platelets, but also on a wide variety of other cell types where it mediates adhesion to collagen and as a consequence makes important contributions to the control and maintenance of cell phenotype. The first real clue to the receptor function of the α2β1 integrin came from platelet pathobiology. Nieuwenhuis et al described a patient with a bleeding disorder that showed selective nonresponsiveness to collagen used as a stimulus for platelet aggregation, markedly impaired adhesion to collagenous substrates, and a selective deficiency of the α2 integrin subunit (glycoprotein Ia) resulting in the absence of platelet surface α2β1 integrin. Subsequently, additional patients have been described with bleeding disorders arising from the development of inhibitory antibodies against the α2β1 integrin or acquired deficiency of the integrin associated with myeloproliferative disorders or other etiologies.

After the development of stable α2β1 integrin-mediated adhesion, glycoprotein VI engages collagen as a lower affinity, signal transducing coreceptor. Although glycoprotein VI has yet to be purified, characterized, or cloned, its role in collagen-induced platelet activation seems relatively clear. In the absence of α2β1 integrin expression or in the presence of effective α2β1 integrin blockade, glycoprotein VI is unable to support effective platelet adhesion to collagen. On the other hand, in the absence of glycoprotein VI expression or in the presence of an inhibitory glycoprotein VI antibody, adhesion occurs (although it is reduced) but subsequent platelet activation is markedly impaired. Most recent studies point to roles for signals emanating from both the α2β1 integrin and glycoprotein...
VI as essential for the attainment of maximal collagen-induced aggregation/activation. In glycoprotein VI-deficient platelets, collagen-induced activation of syk is severely compromised; collagen increased expression of c-src is not. A putative glycoprotein VI ligand stimulates tyrosine phosphorylation of syk and phospholipase Cγ2. These events appear to represent important steps along the pathway to collagen-induced platelet activation. It is not yet clear where the 65-kD protein recently described by Chiang et al fits into the process.

As a consequence of platelet activation and inside-out signaling, glycoprotein IIb-IIIa (αIIbβ3 integrin) acquires the ability to bind fluid phase fibrinogen and thereby produce platelet aggregation. Only when the entire series of ligand-receptor interactions takes place in a concerted and coordinated manner does normal formation of the platelet hemostatic plug occur.

The two articles in this issue draw our attention to previously unrecognized (potential) contributions of platelet surface α2β1 integrin expression to hemorrhagic and thrombotic disorders. One, by Di Paola et al, focuses on the exacerbation of bleeding manifestations in von Willebrand disease associated with lower levels of platelet surface α2β1 integrin. A second by Carlsson et al raises the possibility that higher levels of platelet α2β1 integrin expression constitute a genetic risk factor for the development of stroke in younger patients.

The α2β1 integrin is expressed at relatively low level (only 1,000 to 3,000 copies per platelet) relative to other major platelet adhesive receptors. Although relatively low, the level of platelet α2β1 expression varies widely (up to 10-fold) and correlates with platelet adhesivity to collagens. Recent analyses indicate that DNA sequence polymorphisms within the α2 integrin gene are linked to the level of platelet surface α2β1 integrin expression. The sequence variants include two silent (ie, the amino acid sequence is unchanged) polymorphisms at nucleotide 807 (T or C) and nucleotide 873 (A or G). The two polymorphisms are linked. The 807C/873G pair is associated with lower levels of α2β1 integrin expression than is the 807T/873A pair. The molecular mechanism(s) underlying the differing levels of expression remain to be elucidated.

Although the varying levels of α2β1 expression appear to be of little significance in individuals that are otherwise hemostatically normal, Di Paola et al reasoned that the varying collagen receptor expression associated with varying α2β1 expression might become clinically significant/apparent in a setting where the overall platelet-collagen interaction was already partially compromised. Thus, they chose to study patients with von Willebrand disease. In the setting of mild type I von Willebrand disease they found that for a given level of ristocetin cofactor activity, the time to closure of a model wound was more prolonged in individuals with the 807C polymorphism (low α2β1 expressers) than in individuals with the 807T polymorphism (high α2β1 expressers). Not unexpectedly, in the setting of more severe von Willebrand disease which itself produced a profound defect in closure time, the level of α2β1 integrin expression was of no additional consequence. Thus, varying density of collagen receptor expression on the platelet surface might contribute to the recognized variability of bleeding manifestations observed among von Willebrand disease patients (even within a family) with similar von Willebrand factor antigen and ristocetin cofactor activity levels.

Carlsson et al took a complementary approach. The intriguing results of their small case-control study revealed that the 807T/873G polymorphism (high expressors) was the only over-represented variable (odds ratio, 3.02; 95% confidence interval, 1.20 to 7.61) in a group of young (<50 years) stroke patients. The prevalence of traditional risk factors such as hypertension, diabetes mellitus, smoking, elevated cholesterol, and family history did not significantly differ between the case and control populations. Unfortunately, neither the presence of atherosclerosis nor of other hematologic risk factors was ascertained. In older patients (>50 years) the more traditional risk factors dominated and the 807T/873G polymorphism was not a significant risk factor. Although the finding of this intriguing investigation is intuitively pleasing, because the study is small and the risk relatively modest, it would be premature to trumpet the 807T/873G polymorphism as a newly established risk factor for stroke in the young. However, the early work is tantalizing and certainly merits more study, not only in the setting of stroke, but in the setting of other thromboembolic diseases where platelet reactivity with collagens might be implicated.

These two studies nicely demonstrate the potential relevance of α2 integrin gene polymorphisms and the associated variation in platelet surface α2β1 integrin expression in complex hemorrhagic or thrombotic disorders where the degree of platelet reactivity with collagenous substrates might contribute to the clinical phenotype. The contributions and/or associations of platelet adhesive receptor polymorphisms with complex, multifactorial diseases is a growing and potentially important avenue of investigation.

REFERENCES

Platelet Surface Collagen Receptor Polymorphisms: Variable Receptor Expression and Thrombotic/Hemorrhagic Risk

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