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Neutralize the neutrophils! Neutrophil β_1/β_2 integrin activation contributes to JAK2-V617F-driven thrombosis

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Thrombosis is a major problem for patients with myeloproliferative neoplasms (MPNs). Leukocytes have long been speculated to contribute to thrombotic development in MPNs, but the exact role of these cells has not been fully elucidated. In this issue of the *JCI*, Edelmann and colleagues demonstrate that granulocytes from mice expressing an MPN-associated JAK2 mutation (JAK2-V617F) exhibit enhanced adhesion to VCAM1- and ICAM1-coated surfaces. The increased adhesion was shown to be mediated by β_1 (VLA-4) and β_2 integrins, which are activated via inside-out signaling induced by JAK2-V617F. In a murine thrombosis model, administration of neutralizing antibodies targeting VLA-4 and β_2 integrin reduced thrombosis, suggesting the intriguing possibility that targeting these pathways could have clinical relevance for MPN.

Thrombosis in myeloproliferative neoplasms

Patients with myeloproliferative neoplasms (MPNs) exhibit a propensity for thrombosis, which leads to significant morbidity and mortality (1, 2). Both arterial (e.g., stroke, myocardial infarction) and venous (e.g., pulmonary embolism, deep vein thrombosis) systems can be affected, and unusual locations can be involved, such as the splanchnic vasculature. The prevalence of thrombotic events has been reported to range from 10% to 29% in essential thrombocythemia (ET) and 34% to 39% in polycythemia vera (PV) patients (3). In one population-based study, the incidence of arterial and venous thrombosis in the first 3 months after MPN diagnosis was 3 and 10 times higher, respectively, compared with the incidence in individuals without MPN (4).

Established risk factors for thrombosis in MPNs include older age and prior history of thrombosis (5). As the defining

feature of PV is erythrocytosis, increased RBC mass presumably is a primary factor that drives thrombosis. Thus, current guidelines for PV patients recommend maintaining the hematocrit (HCT) at a level less than 45% (6). The importance of this specific target was validated by a study in which patients were randomized to two different treatment goals (HCT less than 45% versus HCT of 45%–50%) that demonstrated that the lower HCT goal associated with a lower likelihood of death from cardiovascular causes or major thrombotic events (7). In ET, the cardinal feature is excessive platelet production, although the degree of thrombocytosis (i.e., platelet count) has not been shown to correlate well with the risk of thrombosis (2). Current guidelines indicate that ET patients considered at high risk for thrombosis should be treated with cytoreductive therapy (most commonly hydroxyurea) to normalize platelet count (6).

A role for leukocytes in promoting MPN-associated thrombosis?

MPN patients also commonly exhibit leukocytosis, and some studies have implicated leukocytosis as an independent risk factor for thrombosis (8–10). As noted above, cytoreductive therapies, such as hydroxyurea, are commonly used to reduce the HCT and/or platelet count in PV and ET patients. However, it has been speculated that an important benefit of hydroxyurea may be to lower the white blood count (WBC), thereby mitigating a potential contribution of leukocytes to thrombus formation (11). Neutrophils specifically have been recently recognized as integral to thrombus initiation and progression. Proposed mechanisms by which leukocytes could contribute to thrombosis include the release of proteolytic enzymes by activated neutrophils, as well as increased CD11b expression, leading to stronger attachment of leukocytes to the endothelium and platelets (1, 2). Abnormal generation of neutrophil extracellular traps (NETs), which contribute to coagulation and platelet aggregation, has also recently been linked to the MPN-associated mutation JAK2-V617F and thrombosis (12).

β_1 and β_2 integrin activation contributes to JAK2-V617F-mediated thrombosis

β_1 and β_2 integrins are essential mediators of leukocyte adhesion to the endothelium. In this issue, Edelmann and colleagues hypothesized that in MPNs, abnormal integrin function on leukocytes could contribute to thrombus formation (13). Granulocytes isolated from JAK2-V617F knockin mice exhibited increased adhesion to VCAM1 and ICAM1, ligands for β_1 and β_2 integrin, respectively (Figure 1A). These findings are consistent with recent studies from the same group show-

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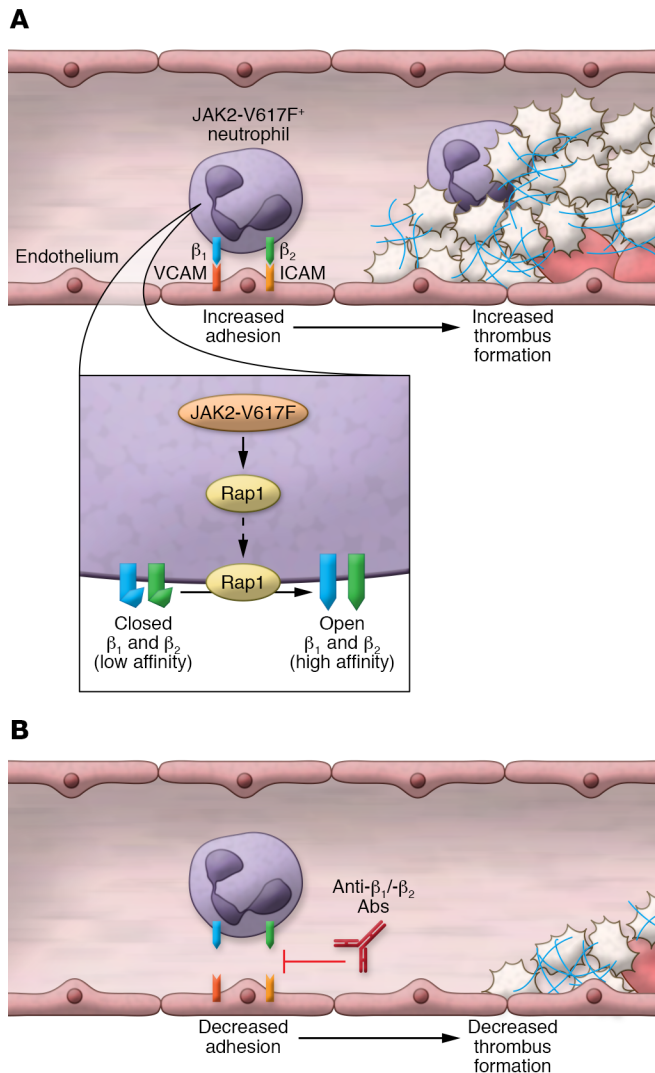


Figure 1. The myeloproliferative neoplasm-associated mutation JAK2-V617 promotes thrombus formation. (A) Neutrophils expressing JAK2-V617 have increased activation of β_1 and β_2 integrin, resulting in increased adhesion to VCAM and ICAM1 on the vascular endothelium and enhanced thrombus formation. JAK2-V617 enhances activation of Rap1, which then translocates to the plasma membrane, thereby inducing the inside-outside signaling that shifts β integrins from a closed, low-affinity conformation to a high-affinity conformation (inset). (B) Antibodies targeting β_1 and β_2 integrin reduce neutrophil adhesion, resulting in decreased thrombus formation.

ing that granulocytes from JAK2-mutant MPN patients have increased adhesion to VCAM1 (14).

Utilizing a conformation-specific antibody for β_1 integrins, Edelmann and colleagues found that JAK2-V617F expression shifts β_1 integrins from a closed, low-affinity conformation to an open, high-affinity conformation (Figure 1A). This conformation change occurred via integrin inside-outside signaling that involves Rap1-GTPase. In granulocytes from calreticulin-mutant (CALR-mutant) MPN patients, Rap1 was activated to a lesser degree than in JAK2-V617F granulocytes. This differ-

ence in Rap1 expression is notable, since CALR-mutant MPN patients are known to be at lower risk for thrombosis compared with JAK2-mutant MPN patients.

Activation of Rap1-GTP is associated with translocation to the plasma membrane, and JAK2-V617F expression was shown to promote Rap1 membrane relocalization. Moreover, GGTI-2147, a geranylgeranyltransferase inhibitor that blocks the posttranslational modifications required for Rap1 activation and translocation to the plasma membrane, inhibited adhesion of JAK2-mutant (but not JAK2-WT) granulocytes to VCAM1. JAK2-mutant

granulocyte adhesion to VCAM1 was also reduced following incubation with the PI3K inhibitor wortmannin. Similar reductions in adhesion were obtained with the Ca^{2+} and Mg^{2+} chelator BAPTA/AM, as well as with knockdown of the Ca^{2+} -dependent enzyme CalDAG-GEFI, which is involved in conversion of Rap1-GDP to Rap1-GTP. Together, these findings suggest a role for PI3K and CalDAG-GEFI signaling in Rap1 activation mediated by JAK2-V617F.

To determine the contribution of β_1 and β_2 integrin activation to JAK2-V617F-induced thrombosis Edelmann et al. utilized an inferior vena cava (IVC) ligation model. Compared with JAK2-WT mice, JAK2-mutant mice exhibited a significant increase in thrombus size in response to partial IVC ligation that was dramatically reduced by injection of β_1 and β_2 integrin-blocking antibodies (Figure 1A). These findings indicate that β_1 and β_2 integrins play an important role in JAK2-V617F-driven thrombosis, and suggest that targeting β_1 and β_2 integrin activation could potentially be efficacious clinically.

Perspective

Together, these studies by Edelmann and colleagues highlight the role of neutrophils in MPN-associated thrombosis and shed light on the mechanism by which JAK2-V617F activates β_1 and β_2 integrins to promote thrombus formation. One outstanding question not addressed in this study is whether inhibition of JAK2 with agents, such as ruxolitinib, might impact β_1 and β_2 integrin activation driven by mutant JAK2. Regardless, the studies shown establish that blocking β_1 and β_2 integrin activation could mitigate JAK2-V617F-driven thrombosis. Agents that target various integrin subunits have shown promising activity in several inflammatory diseases, including multiple sclerosis and inflammatory bowel disease, although, integrin-targeting therapies have been associated with increased susceptibility to infection (15, 16). Whether targeting β_1 and β_2 integrin activation as a means of preventing thrombosis in MPNs is a viable strategy merits further exploration.

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