African ancestry. Such differences in white blood cell (WBC) count could clearly impact on disease course.

Ahuja and colleagues previously reported that DARC-null individuals show increased susceptibility to HIV infection, but slower HIV disease progression once infected. In this issue of Blood, Kulkarni et al examine associations among WBC count, DARC genotype, and survival in a large natural history cohort of HIV-infected Americans of both European (EA) and African (AA) descent. Leukopenia, defined as an average WBC count during disease of less than 4 × 10⁹ cells per liter, was associated with faster HIV disease progression rates, but notably leukopenic EAs had a slower disease course than leukopenic EAs. As in uninfected EAs, the DARC-null state was associated with lower WBC count in HIV⁺ AAs. Significantly, among leukopenic subjects, there was a survival advantage for DARC-null AAs compared with DARC-positive AAs or EAs. By contrast, rates of disease progression in nonleukopenic AAs did not differ by DARC genotype. Although the mechanism(s) underpinning the survival advantage in DARC-null leukopenics remains unclear, several thought-provoking possibilities are aired in the article’s discussion. It should be noted, however, that several recent studies failed to find associations, in other cohorts, between DARC genotype and and an individual’s susceptibility to HIV infection or disease progression. Possible explanations for these discrepancies have been discussed previously by Ahuja and colleagues. One of these explanations is highlighted in this new report by Kulkarni et al. It suggests that it is the strong interaction between DARC genotype and WBC counts that influences HIV disease course. Further studies are required to determine, for example, whether similar interactions influence HIV susceptibility, and how DARC genotype and WBC counts are mechanistically linked. Nonetheless, the novel functions emerging for DARC in homeostasis and disease mean that we are steadily making progress toward the heart of DARCness, and it is clearly going to be an exciting and illuminating journey.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES


Platelet integrin signaling: wherefore art thou?

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In this issue of Blood, Mazzucato and colleagues demonstrate that collagen receptors GPVI and α2β1 integrin generate qualitatively distinct, independent calcium signals during platelet tethering and adhesion to collagen under flow conditions. Rapid-spiking, short-lived, calcium signals using intracellular calcium stores are generated by α2β1 signaling in a glycoprotein (GP)VI-independent fashion. GPVI is responsible for sustained calcium mobilization dependent on extracellular calcium influx but requires the presence of α2β1 signaling. These findings add complexity to our understanding of how α2β1 and GPVI interact in response to collagen and provide additional evidence that platelet activation and platelet adhesion are not distinct and independent events.

Collagen-induced platelet adhesion and activation under flow conditions are considered primary events in the formation of the intra-arterial thrombi that cause stroke and heart attack. Studies in the past decade have identified 2 platelet collagen receptors, the immune-type receptor GPVI and the integrin α2β1, as critical for the formation of platelet thrombi on collagen under flow ex vivo and for arterial thrombus formation under some circumstances in vivo. A widely accepted model of platelet collagen responses assigns GPVI the role of generating platelet activation signals, including “inside-out” signals required to conformationally activate integrins on the platelet surface, and α2β1 the role of mediating firm adhesion to collagen in the face of powerful shear forces. This model is supported by genetic and pharmacologic loss-of-function studies demonstrating that GPVI signaling is required for platelet activation by collagen, a prerequisite for platelet adhesion to collagen under flow. In contrast, α2β1 is required for platelet adhesion to collagen under flow but not for platelet activation by collagen.

This clean division of labor suggested by loss-of-function studies has been challenged by findings that have identified roles for α2β1 in the generation of intracellular signals that might initiate or prolong platelet activation. A significant report in the literature, mostly focused on αIβb3, supports “outside-in” signaling by integrins through the same pathway used by GPVI. The ability of α2β1 to activate this pathway has been demonstrated and, like GPVI, uses Src-family kinases, Syk, SLP-76, and PLCγ2. However, unmasking a role for α2β1 signaling in physiologic platelet-collagen responses has been difficult because it
shares a common ligand with GPVI and requires previous activating signals to adopt a high-affinity conformation needed for collagen adhesion. Mazzuccato et al make an important contribution by showing that, as platelets interact with collagen under flow, α2β1 receptors generate calcium signals that are distinct from those generated by GPVI. Surprisingly, α2β1-dependent calcium signals are even generated in the absence of GPVI, forcing us to re-examine the concept of strict dependence on previous inside-out integrin activation for α2β1 ligand binding and whether GPVI is a major source of these inside-out signals. Even more unexpected is the finding that GPVI-associated calcium signals do not take place in the absence of α2β1, suggesting a stronger synergy between α2β1 and GPVI than previously appreciated. These intriguing results are significant because they document integrin-dependent signals during the actual process of platelet interaction with collagen under flow, a response believed to faithfully reproduce the in vivo interaction between platelets and vessel wall collagen.

A major question raised by this and previous studies is the functional importance of these integrin-generated signals. Various studies have suggested that α2β1 can synergize with GPVI during platelet collagen responses, but defining a direct signaling role for α2β1 has been more difficult. Mazzuccato et al note that more than half of platelets lack functional GPVI.12 Much of the increased calcium signals, and GPVI-deficient platelets do not exhibit robust collagen signaling despite the presence of α2β1 integrins. Thus, one possibility is that these integrin signals are either not functionally significant or not sufficient to support platelet activation. Alternatively, as suggested by the authors, the signals generated by GPVI and α2β1, like similar signals generated by GPIbα and integrin αIIbβ3 during platelet rolling on von Willebrand factor, may function in an interactive and even reciprocal fashion as platelets interact with matrix ligands such as collagen that are both activating and adhesive. Such interdependent molecular interactions are particularly difficult to unravel using standard loss-of-function experiments, and a more detailed understanding of molecular mechanisms by which integrins trigger such signaling events will be needed to test their biological roles.

Conflicts of interest: The authors declare no competing financial interests.

References

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Super factor B—gets atypical HUS

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Roumenina and colleagues identify mutations in complement factor B that render it uniquely hyperfunctional, predisposing affected persons to atypical hemolytic uremic syndrome and revealing novel mechanistic insights.

A typical hemolytic uremic syndrome (aHUS) is a rare but devastating disorder, characterized by thrombocytopenia, microangiopathic hemolytic anemia, and renal dysfunction. More than 50% of patients develop end-stage renal failure and 25% die during the acute phase of the illness.1 Recent studies indicate that the hallmark aHUS-associated microvascular and arteriolar endothelial cell injury is pathogenically linked to excess complement activation via the alternate pathway (AP). This is supported by the finding that approximately 50% of patients have disease-associated, AP complement-activating missense mutations in genes encoding complement regulatory proteins (factor H [FH], factor I, membrane cofactor protein, factor H–related protein, C4b binding protein, thrombomodulin, C3, and factor B [FB]).2,3 Despite these insights, therapies are lacking, and several key questions remain: What is the etiology of the remaining 50% of cases? Why is there variable penetrance of the syndrome? What are the triggers for onset? Why is the endothelium targeted? What precipitates the thrombosis? Why is the kidney most frequently and severely affected?

Genetic studies of patients with aHUS, coupled with a greater understanding of the molecular mechanisms of complement regulation, are providing some answers. The AP provides a rapid route for complement activation and amplification on the surfaces of invading pathogens (see figure, panel A).4 Initiation occurs spontaneously with hydrolysis of C3 to form metastable C3(H2O) to which the plasma protein FB may bind in solution. FB is then susceptible to cleavage by the circulating protease, factor D (FD), exposing a serine protease domain on the Bb fragment of FB. The so-formed fluid-phase C3 convertase, C3(H2O)Bb, cleaves additional C3 into C3a
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