to reveal patterns of gene reactivation that characterized responders and nonresponders. Given that aza-nucleosides are known to induce DNA damage, Fandy et al also determined the levels of γH2AX, a well-known marker of DNA damage, in their patient samples. γH2AX is a phosphorylated form of the histone variant H2AX that becomes rapidly and specifically localized to regions of DNA containing double strand breaks. This aspect of aza-nucleosides has not been examined in earlier studies and, in fact, revealed a robust induction of γH2AX even at the lowest doses of 5-azaC. Like the other parameters examined in this study, however, there was no correlation between γH2AX induction and clinical response as it occurred in responders and nonresponders.

So where do we go from here with epigenetic-based cancer therapies? Although there is little doubt the aza-nucleosides induce demethylation in patient tumor cells, the exact therapeutic molecular target(s) remains an open question. At the most simplistic level, one could argue that we simply have not found the one or few critical genes that, when demethylated and reexpressed, result in differentiation or cell death. For these studies, use of more sensitive microarray platforms than the one employed by Fandy and colleagues, along with more quantitative confirmatory methods such as quantitative RT-PCR will likely help to address this possibility. Coupling these methods with genome-wide tiling arrays or high-throughput sequencing DNA methylation analyses would also be expected to shed significant light on whether a few critical genes are being targeted by epigenetic therapies. Compared with cytotoxic combination chemotherapy, the slower development of response occurring after 4 cycles of therapy may suggest that the epigenetic effects and ensuing potential differentiation/cytotoxic effects were occurring in the small subset of early progenitor or stem cells. Therefore, as much as possible, the correlative analyses should be performed in these cells rather than in the bulk progenitors. Other parameters worthy of future consideration that could influence or predict clinical responses to epigenetic therapies include DNMT expression levels and expression of modifiers of aza-nucleoside bioavailability: nucleoside transporters like hCNT1 or cytosine deaminase. Finally, it should be considered that aza-nucleosides and HDAC inhibitors are exerting effects indirectly on other epigenetic marks, such as histonemethylation, and that these may be more predictive of clinical response. It is well known that DNA methyltransferases interact not only with HDACs but also with various histone methyltransferases and, as such, it is reasonable to expect that interfering with one epigenetic mark will impact other marks. The full extent of this interaction in vivo in human patients has yet to be explored.

Overall, this study creates a sobering realization about our lack of full understanding of several important dimensions of epigenetic targets and therapies. Identifying the predictive biomarkers of clinical response and elucidating the mechanisms of resistance to epigenetically targeted agents looms as a big challenge, but a compelling necessity for learning and refining the steps of this tango!

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REFERENCES

 Comment on Kulkarni et al, page 2783

HIV: getting to the heart of DARCness

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In this issue of Blood, Kulkarni and colleagues continue their exploration of genetic regulation of HIV pathogenesis, and report that the absence of erythrocyte Duffy antigen is associated with a survival advantage in leukopenic, but not nonleukopenic, HIV-infected African Americans.1 C hemokines and their receptors play critical roles in HIV pathogenesis, and genetic variation in the chemokine system has a profound impact on susceptibility to HIV infection and progression to AIDS. Ahuja’s group and their collaborators have made key contributions to these genetic studies, and their recent work2 has led to increased attention to the role of the Duffy antigen, or DARC (Duffy antigen receptor for chemokines), in HIV pathogenesis. DARC is a nonsignaling chemokine receptor that, on erythrocytes, regulates the abundance of proinflammatory chemokines in serum.3 It also acts as a docking site for the malarial species Plasmodium vivax, and this is believed to have driven the emergence of the DARC-null state (ie, erythrocytes lacking DARC) common among persons with sub-Saharan African ancestry.4 DARC could impact on HIV pathogenesis in a number of ways. First, DARC binds several of the chemokine ligands for CCR5, the critical coreceptor for HIV entry into cells. These chemokines can suppress HIV entry, so their regulation by DARC could modify cellular infection rate. Second, erythrocyte DARC binds HIV where it remains viable for infection of CCR5+ T cells.5,6 Third, the DARC-null state was recently shown, for reasons that remain unclear, to be the principal genetic determinant for the benign ethnic leukopenia (primarily neutropenia) seen in people of sub-Saharan African ancestry.4
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 AAs had a slower disease course than leukopenic EAs. As in uninfected AAs, the DARC-null state was associated with low 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 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.

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PLATELETS & THROMBOPOIESIS

Comments on Mazzucato et al, page 2793

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 αIibβ3, 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 PLCg2. However, unmasking a role for α2β1 signaling in physiologic platelet-collagen responses has been difficult because it
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