after therapy in each case, 3 very different temporal patterns of repopulation were observed. One patient revealed stable equilibrium of 5 subpopulations over the course of years, despite therapy (see figure panel A). In contrast, a separate patient demonstrated the near complete replacement of the tumor after cytotoxic therapy by a subclone that was originally only a small subpopulation at an earlier time point (panel B). Finally, the third patient demonstrated a hybrid pattern—an initial stable balance between subclones that over time led to expansion of 1 subclone that became the dominant subclone at the time of the patient’s death.

What do these patterns tell us? Although only 3 cases were analyzed, the results of sampling multiple serial time points per patient suggest the presence of dynamic forces that can impact the stability of CLL genetic composition over time. For example, the scenario provided in panel A suggests the presence of clonal interference—that co-existing subclonal populations may limit each others’ growth by mutual competition. In contrast, a separate insight is suggested by the scenario illustrated in panel B, in which a more aggressive clone emerges after therapy that was present in the pretreatment sample at low frequency (detectable by deep sequencing). On one hand, this could be explained by the active selection of particular genetic alterations by therapy. Alternately, harkening to paradigms formulated in evolutionary biology, cytotoxic therapy could act as a classic evolutionary restriction point and reset interclonal dynamics in favor of a more aggressive clone by markedly reducing disease bulk and by removing the incumbent clone.50

These data invite one to speculate that the composition of CLL of any particular individual results from personal “ecology”—a result of the interplay between both intrinsic (eg, inherent “driverness” among subclones) and extrinsic/environmental factors (eg, time since transformation, exposure to cytotoxic therapy). The work by Schuh et al thus serves as an important starting point, and inspires numerous questions. How frequently are the patterns of interclonal equilibrium or of clonal evolution observed in patients and how do they relate to known prognostic risk factors in CLL? Do these patterns hold prognostic or predictive significance? What are the underlying genetic factors that determine whether subclones reseed or expand? Can identification of subclonal mutations before therapy anticipate the composition of the relapsing tumor? Answering each of these questions will require tumor sequencing of larger and preferably uniformly treated patient sample sets along with an analysis pipeline that can accommodate these large datasets. In turn, addressing these questions has the potential to reveal whether the presence of subclonal populations actually impacts CLL disease tempo or responsiveness to therapy.

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

REFERENCES

●●● PLATELETS & THROMBOPOIESIS

Comment on Sadoul et al, page 4215

The spreading influence of platelet HDACs

Benjamin T. Kile1 1THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH

In this issue of Blood, Sadoul et al demonstrate that histone deacetylase 6 (HDAC6) mediates platelet microtubule deacetylation in response to activation.1

The production of platelets by megakaryocytes requires an extraordinary feat of cellular remodeling. The tubulin cytoskeleton is central to this process. It provides the structural basis for proplatelets: long, filamentous extensions of megakaryocyte cytoplasm that bend and branch, projecting their tips into sinusoidal blood vessels so that platelets may bud off into the circulation.2 Considerable progress has been made in elucidating the biology of proplatelets. We now understand that their formation and maturation is a highly dynamic process, and that proplatelet behavior dictates both the form and—via organelle trafficking—the content of platelets. Recent work has yielded insights into the terminal stages of platelet biogenesis in vivo, indicating that rather than release perfectly formed platelets one by one, megakaryocytes shed large fragments of proplatelets, termed preplatelets, into the bloodstream.3 The latter undergo maturation and abscission to generate individual platelets.4

As a major structural component of proplatelets, tubulin therefore plays a critical role in platelet production. This is illustrated by the demonstration that deletion of the megakaryocyte-specific β-tubulin isoform β1 in mice results in a failure of proplatelet formation and subsequent thrombocytopenia.3 The regulation of the tubulin network within the megakaryocyte lineage, however, remains something of a mystery. This is particularly true of platelets, whose characteristic discoid shape is defined and maintained by a marginal band of microtubules. In response to activation, platelets undergo dramatic shape changes, involving a major reorganization of the tubulin cytoskeleton. Here, in an elegant series of experiments, Sadoul and colleagues demonstrate that the latter process is accompanied by profound changes in the acetylation

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status of microtubules. Within minutes of human platelets being allowed to spread on glass coverslips, tubulin was almost completely deacetylated. Over the course of several hours, as platelets began to retract, reacetylation occurred. The same phenomenon was observed when platelets were spread on glass coated with fibronectin, fibrinogen, or collagen, or induced to aggregate with arachidonic acid, adenosine diphosphate, or collagen.

Acetylation is one of a number of post-translational modifications (PTMs) that occur on microtubules, including deacetylation, glutamylation, and glycylation. These PTMs are thought to regulate stability and influence microtubule-based functions such as motor protein movement. While the marginal band of platelets is known to be highly acetylated, Sadoul et al's work is the first indication that microtubule acetylation in platelets is dynamically regulated. Deacetylation occurred in response to microtubule depolymerization induced by nocodazole, but not stabilization induced by taxol, suggesting that the process is driven by a deacetylase gaining access to its substrate. This would fit with the structural orientation of the major site of acetylation, lysine 40 of the N-terminal domain of α-tubulin, which is predicted to reside on the luminal face of microtubules.

But which enzyme is responsible for deacetylation? After comparing the effects of different classes of deacetylase inhibitor, Sadoul and colleagues narrowed their focus to HDAC6, which along with Sirtuin 2 is known to be a key tubulin deacetylator. Examining platelets from HDAC6-deficient mice, they found that, at steady state, the marginal band was hyperacetylated relative to wild-type counterparts. HDAC6-deficient platelets was hyperacetylated relative to wild-type platelets, exhibiting no changes in microtubule deacetylation. Thus, HDAC6 is essential for microtubule deacetylation upon platelet activation.

How important is this process to platelet function? Sadoul et al show that platelets lacking HDAC6 spread more rapidly than wild-type controls, although the difference is transient, with no significant differences in spread morphology evident after 90 minutes. Therefore, while HDAC6-mediated deacetylation of microtubules is not absolutely required for platelet spreading, it does shape the kinetics of the response. The major question arising from the current study is whether this translates to defects in hemostasis and/or thrombosis in vivo. Platelet spreading in vitro is a valuable readout of platelet function, but the phenotype of HDAC6-deficient platelets is subtle, and it will be important to understand the nature of the physiologic role that tubulin de- and reacetylation plays. This is particularly salient given that a number of histone deacetylase inhibitors—some of which target HDAC6—are in clinical development, primarily in the context of cancer therapy.

Several of these agents are associated with thrombocytopenia, and recent evidence suggests this is due to their impairment of cytoskeletal dynamics in megakaryocytes. Whether they might also interfere with the function of platelets remains to be addressed.

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●●● THROMBOSIS & HEMOSTASIS

Comment on Pötschke et al, page 4238

HIT and run: heparin’s unusual immune response

Gowthami M. Arepally1

In this issue of Blood, Pötschke et al investigate the cellular underpinnings of the heparin-induced thrombocytopenia (HIT) immune response. The immune response to heparin is peculiar. Unfractionated heparin (UFH) and its derivatives, the low-molecular weight heparins (LMWHs), are essential for initiating HIT, one of the most common and serious drug-dependent responses in hospitalized patients. In certain clinical settings, such as cardiac surgery, seroconversion is common-place (~ 50%), but clinical manifestations of disease are, fortunately, less frequent (1%-2%). Although the antibodies causing HIT recognize heparin complexed to an endogenous platelet protein, platelet factor 4 (PF4), heparin is not necessary to carry out the dire business of the antibodies as cell-surface glycosaminoglycans (GAGs; heparan sulfate or chondroitin sulfate) can readily substitute for the drug in the PF4/heparin antigenic complex.

Another peculiar aspect of the HIT immune response is its serologic transience. Given the abundance of antigenic material in the host in the form of PF4 and cell-surface glycosaminoglycans, one would naturally expect the PF4/heparin immune response, once it occurs, to be self-sustaining. Yet, thankfully, it is not. The immune transience of HIT is all the more striking, as recent studies indicate that bacterial infection and trauma may serve as “danger” signals to predispose individuals to become sensitized. These unusual serologic features of HIT remain largely unexplained.

To understand why HIT is so atypical, Pötschke and colleagues undertook studies to
The spreading influence of platelet HDACs

Benjamin T. Kile