Nonetheless, the activation of multiple PP2A complexes bodes well for the therapeutic efficacy of the sphingosine analogs.

While PP2A activation has been proposed as a therapeutic option for a range of cancers with inactivated PP2A, clinical trials have yet to eventuate. The demonstration that a nonphosphorylatable sphingosine analog, OSU-2S, is effective at eradiating AML stem cells while sparing normal immune cells is highly encouraging for clinical translation, and human trials are eagerly awaited.

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

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THROMBOSIS AND HEMOSTASIS

Comment on Hur et al, page 1374

Fibrinogen levels and thrombosis prevention

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In this issue of *Blood*, Hur et al[1] expand our understanding of hypodysfibrinogenemia, where a naturally occurring variant (fibrinogen Otago) exhibits normal hemostatic and antimicrobial functions, while protecting against thrombosis, in mice (see figure).

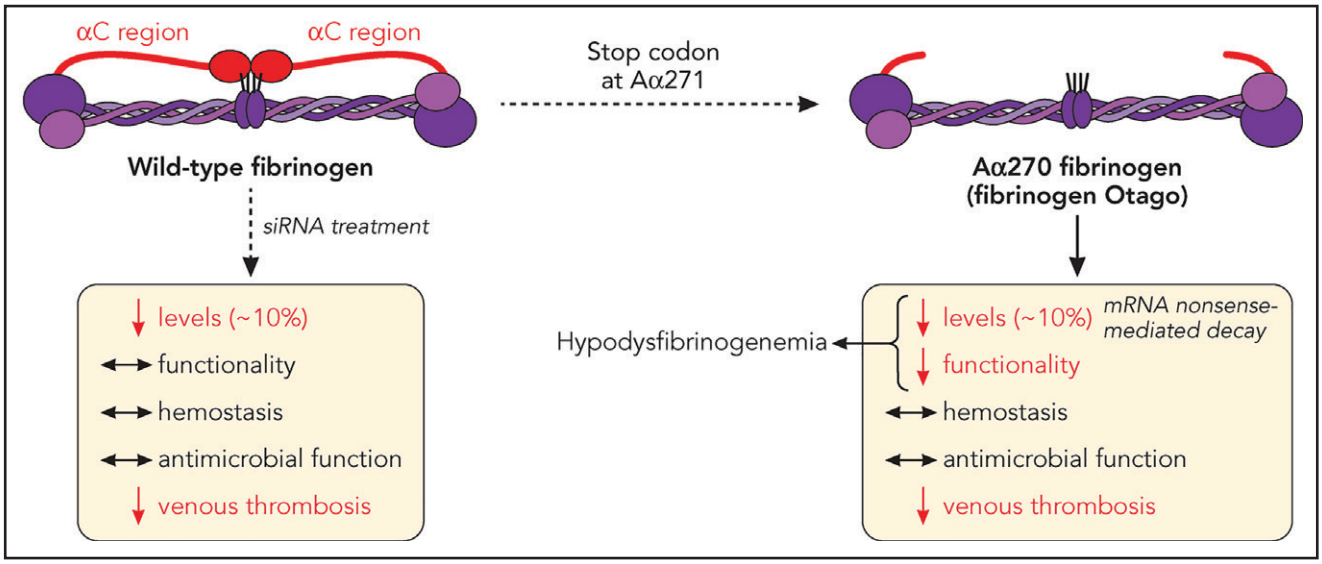
Congenital hypodysfibrinogenemia is characterized by reduced plasma levels of fibrinogen alongside altered function, mostly caused by mutations in the fibrinogen Aα chain.[2] However, the mechanisms underpinning the reduced circulating fibrinogen levels, and the relevance of inherent effects of the mutation vs those of the reduced fibrinogen levels on the hemostatic/thrombotic balance, are still unknown.

On one hand, afibrinogenemia (no circulating fibrinogen) is associated with severe spontaneous bleeding from all tissues, ranging from umbilical cord to the central nervous system, with potentially fatal intracranial hemorrhage, although hypofibrinogenemia (low levels of circulating fibrinogen) is usually asymptomatic in its mild and moderate forms, with virtually no unprovoked bleeding and normal pregnancy.[2] On the other hand, dysfibrinogenemia (abnormal form of circulating fibrinogen), although mostly asymptomatic, presents with risks of developing major bleeding and/or cardiovascular events.[3] The question remains as to whether levels of circulating fibrinogen are a driving force to several pathological conditions. In contrast to afibrinogenemia and hypofibrinogenemia exhibiting bleeding tendencies, elevated circulating fibrinogen levels are associated with increased risk of cardiovascular disease, such as coronary heart disease and stroke.[4] Paradoxically, afibrinogenemic patients[2] and fibrinogen-deficient mice[5] present with an increased risk of thromboembolism, attributed to embolization of unstable platelet clots not stabilized by fibrin.

In their article, Hur and colleagues demonstrate that reduction of fibrinogen level to ∼10% of normal circulating levels is sufficient to maintain hemostasis and resistance to infection, while convincingly reducing thrombosis (see figure).

First, the authors describe a transgenic mouse strain (Fga^{270}) replicating a naturally occurring mutation (Otago, fibrinogen Aα chain [Fga] truncation at residue 271), to unravel the mechanisms by which mutations to the fibrinogen Aα chain lead to reduced circulating plasma levels. The Fga^{270} phenotype is similar to that of human hypodysfibrinogenemia and provides the first concrete evidence that nonsense-mediated decay is responsible for decreased levels of protein expression, because treatment of Fga^{270} hepatocytes with cycloheximide increased Fga messenger RNA levels.

Next, the authors showed that small interfering RNA (siRNA) injection in wild-type mice ($Fga^{10\%}$) reduced circulating levels of normal fibrinogen to that of Fga^{270} mice (∼10%), therefore allowing to dissect the impact of low level, vs mutant form, of fibrinogen on the hemostatic/thrombotic balance. Here, low levels of fibrinogen, with(out) Fga mutations, was sufficient to sustain a normal bleeding phenotype, despite Fga^{270} mice forming clots that are less dense with thicker fibers, with both Fga^{270} and $Fga^{10\%}$ plasma exhibiting impaired clotability. To explain this discrepancy, some compensatory mechanisms were proposed, where fibrinolysis was reduced in abnormal Fga^{270} plasma clots, because of the loss of the Aα271-610 region,



Fibrinogen α-chain truncation at Arg_{271} leads to a shortened αC region, a naturally occurring mutation (fibrinogen Otago) leading to hypodysfibrinogenemia (reduced levels and function). This mutation does not dramatically affect hemostasis (bleeding) or fibrinogen-mediated survival to microbial infection, but reduces the development of venous thrombosis, in mice. Similarly, siRNA-induced reduction of circulating fibrinogen levels to ~10% retain fibrinogen functionality, hemostatic and antimicrobial functions, while still preventing venous thrombosis. mRNA, messenger RNA. Professional illustration by Patrick Lane, ScEYEnce Studios.

which is required for the binding of fibrinolytic proteins, while Fga^{270} and $Fga^{10\%}$ platelets exhibited preserved platelet-fibrinogen interaction leading to normal platelet aggregation but faster disaggregation. The normal bleeding phenotype in Fga^{WT}, Fga^{270}, and $Fga^{10\%}$, vs the lack of hemostasis in Fga-deficient mice, is in agreement with studies in hypofibrinogenemic patients showing that those with circulating fibrinogen levels >1 mg/mL (normal range, 2 to 4 mg/dL) remained asymptomatic, whereas decreasing fibrinogen levels correlated with increasing bleeding severity.[6]

Importantly, an inferior vena cava ligation model used in this article demonstrates that both Fga^{270} and $Fga^{10\%}$ mice are protected against venous thrombosis, similarly to Fga-deficient mice, attributing these findings to the lack of fibrin Aα-chain cross-linking responsible for red blood cell retention in the clot and the (lack of) circulating fibrinogen levels, respectively. The treatment of choice for afibrinogenemic patients is prophylactic long-term fibrinogen supplementation with plasma-derived fibrinogen concentrate, with some of those patients found to develop thrombosis following infusion. This study potentially indicates that supplementation toward a lower range of circulating fibrinogen may reduce the risk of thrombosis in those patients.

In addition to hemostasis and thrombosis, fibrinogen also plays a role in host defense against pathogen infection, where afibrinogenemic patients exhibit abnormal skin test reactions to several microbial antigens,[7] and fibrinogen-deficient mice display reduced survival to bacterial infection.[8] Here, Hur and colleagues use a *Staphylococcus aureus* peritoneal infection model to demonstrate that regardless of the fibrinogen Aα chain truncation, a reduction to 10% circulating fibrinogen levels is sufficient to sustain antimicrobial functions, because fibrinogen-mediated bacteria clearance and host survival remained unaffected.

Although this study provides insights into the mechanisms leading to reduced levels of fibrinogen in hypodysfibrinogenemia related to Aα-chain mutations, and some evidence that partial reduction of fibrinogen levels may provide antithrombotic effects without affecting risks of bleeding, the situation is likely more complex. Hence, further investigations should be carried into (1) the role of reduced fibrinogen levels on (i) rebleeding events, (ii) arterial thrombus formation, (iii) thromboembolization in both the venous and arterial systems; (2) the highest fibrinogen levels threshold required to still observe normal hemostasis and reduced thrombosis risks.

Although elevated fibrinogen levels are associated with neurological disorders, such as Alzheimer disease and vascular dementia,[9] a link between fibrinogen levels and cancer is also emerging, with fibrinogen-deficient mice showing reduced primary colon tumor growth.[10] Therefore, the model of reduction in circulating fibrinogen levels presented in this study may also offer opportunities to further define the role of fibrinogen in other diseases.

Lower therapeutic doses of plasma fibrinogen concentrate, and fibrinogen-lowering drugs, have therefore therapeutic potentials for the treatment of congenital fibrinogen disorders and the prevention of cardiovascular diseases and others, respectively.

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

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TRANSPLANTATION

Comment on Adams et al, page 1389

Chronic GVHD of the CNS

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In this issue of *Blood*, Adams et al[1] present a novel mouse model of central nervous system (CNS) chronic graft-versus-host disease (GVHD) and identify unique mediators of this understudied complication after allogeneic hematopoietic stem cell transplantation (HSCT).

Allogeneic HSCT is a curative treatment choice for a wide variety of hematological malignant and nonmalignant diseases. As many as 60% of adult HSCT survivors develop symptoms related to neurocognitive dysfunction.[2] Even though studies have shown that patients with GVHD have increased risk of neurocognitive dysfunction,[3,4] it is currently controversial whether the CNS is a direct target organ of GVHD in humans.[5,6] Because many drugs used in conditioning regimens, immunosuppressants, and infections of the CNS are associated with increased risk of neurocognitive toxicities, it has been difficult to distinguish the direct effects of GVHD on the CNS from those resulting from other complications of HSCT.[3]

Previously published data from both murine and nonhuman primate models of acute GVHD showed that alloreactive T cells could infiltrate the CNS and cause neuronal damage.[7,8] Using multiple tests, Adams et al detected significant behavioral changes in mice with chronic GVHD compared with GVHD-free controls in 2 different models. Like acute GVHD, increased mobility deficits as detected by the forced swim test and learning deficits as revealed in active place avoidance tasks were observed in mice with chronic GVHD. However, the impaired recognition memory, reduced exploratory behavior, and increased anxiety observed in mice with acute CNS GVHD[8,9] were not detected in mice with chronic GVHD.

Behavioral changes could be due to inflammation in the CNS associated with chronic GVHD, as shown in the study by Adams et al. It was observed that T-cell infiltration in the brain was initially dominated by $CD8^+$ T cells and slowly transitioned to $CD4^+$ T cells over time in chronic GVHD. Instead of increased expression of tumor necrosis factor (TNF) and interleukin-6 during acute GVHD,[8,10] persistent upregulation of interferon-γ (IFN-γ) and CC motif chemokine ligand 2 was observed in brains of mice with chronic GVHD. Flow cytometric and immunofluorescent analyses revealed that transient microglia cell activation and infiltration of donor major histocompatibility complex (MHC) class II–expressing macrophages were 2 major characteristics of chronic CNS GVHD. RNA sequencing analyses of donor bone marrow–derived macrophages and host microglia from chronic GVHD brains demonstrated distinguishable transcriptional profiles of these 2 cell subsets. Whereas host microglia returned to a homeostatic state, donor bone marrow–derived macrophages were still highly activated 70 days after transplantation. Of interest, genes related to IFN-γ signaling were highly upregulated in donor-derived macrophages but not host microglia. Decreased neuroinflammation and behavioral changes observed in recipients of MHC class II knockout stem cell grafts suggest that MHC class II expression in donor macrophages plays a critical role in the development of chronic CNS GVHD. Even though the critical role of IFN-γ in chronic CNS GVHD should be further investigated by genetic and pharmaceutical approaches, the data presented in this study strongly suggest that IFN-γ is a mediator of MHC class II expression by donor-derived macrophages. The finding that donor bone marrow macrophages are the culprit behind chronic CNS GVHD is in stark contrast to the situation in acute GVHD of the CNS, in which effect cytokines (eg, TNF) are produced by host microglial cells.[8]

This is a timely report, because we are just beginning to appreciate the effect of alloresponses on the CNS. The unique findings associated with chronic GVHD as revealed by Adams et al[1] (see figure) suggest that the pathogenesis of the CNS damage in chronic GVHD is different from that in the acute setting. Even though a significant amount of work will be needed for us to understand how exactly these immune attacks lead to synapse damage and neurocognitive dysfunction, the data presented in this report lay the foundation for future studies of chronic CNS GVHD.

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