Check for updates

blood commentary

THROMBOSIS AND HEMOSTASIS

Comment on Hur et al, page 105

Better with poorly performing fibrin(ogen)

Li Zhang | University of Maryland School of Medicine

In this issue of *Blood*, Hur et al[1] demonstrate that nonpolymerizable fibrinogen, Fga^{EK}, can preserve hemostatic function without promoting occlusive thrombosis. These findings shed new light on the role of fibrin and fibrin polymerization in thrombosis and hemostasis, with potential implications for developing safer therapeutic agents for thrombotic diseases.

Fibrin clots form through thrombin-mediated conversion of fibrinogen into fibrin (see figure).[2] In this process, thrombin enzymatically cleaves FpA and FpB from fibrinogen, exposing "A" knobs (GPR; high affinity) and "B" knobs (GHR; low affinity) at the N-termini of the α and β chains located within the central E region. These "A" and "B" knobs subsequently interact with holes "a" and "b" in the γ- and β-nodules, respectively, resulting in the polymerization of monomeric fibrin into an extensive fiber network. Activated coagulation factor XIII (FXIII) crosslinks the nascent fibrin fiber, bolstering its stability and resistance to fibrinolysis. This dense fibrin clot seals the damaged tissue and halts blood loss. Once bleeding is under control, fibrin recruits inflammatory cells, initiates the fibrinolytic pathway, and contributes to the wound-healing process.[2] In both human and mice, complete lack of fibrinogen leads to severe bleeding, lack of platelet aggregation, absence of FXIII activation, delayed wound healing, and an elevated risk of perinatal death, as reported in $Fga^{-/-}$ mice.[3]

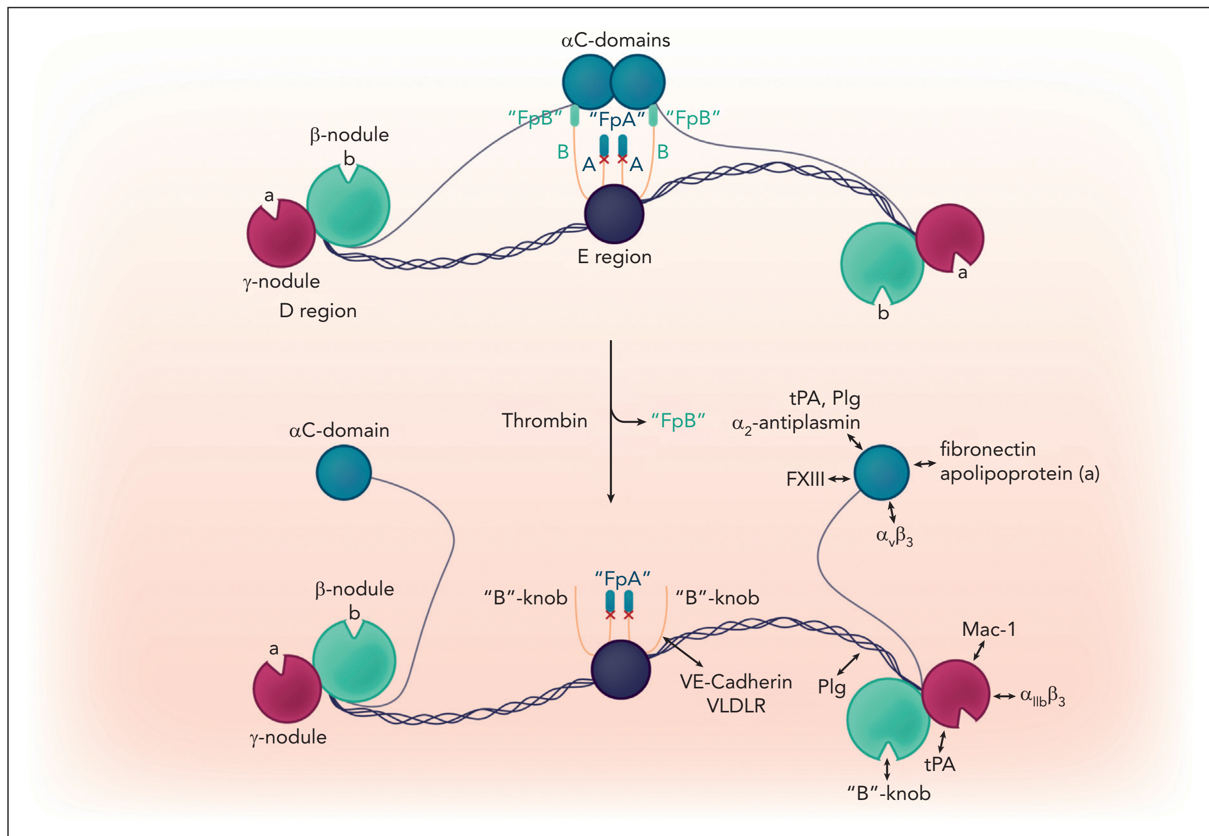
Given the vital role of fibrin polymerization in hemostasis and wound healing, the findings by Hur et al revealing that Fga^{EK} mice, which are incapable of generating polymerized fibrin, display normal hemostasis, platelet aggregation, and wound-healing responses are surprising. Several factors may contribute to the ability of Fga^{EK} to preserve hemostasis and wound healing. Although Fga^{EK} cannot support fibrin polymerization, it contains a functional FpB that can be cleaved by thrombin. Once the "B" knob is exposed, Fga^{EK} can form multimeric complexes through the "B":"b" knob-to-hole interaction and the intermolecular αC-domain:αC-domain interaction as well as support platelet aggregation through γ404-411 and FXIII activation through the αC-domain.[2] Hence, the results from this report support the notion that fibrin, rather than fibrin polymerization per se, is necessary for maintaining hemostatic and wound-healing responses, as long as the "B" knob remains functional.

Fibrinogen is believed to exist in an inert state with low reactivity in light of its high circulating concentration (2-4 mg/mL). Thrombin-mediated removal of FpA and FpB transforms fibrinogen into a reactive molecule capable of interacting with various protein and nonprotein ligands, including the αC-domain of another fibrin(ogen), tPA, plasminogen (Plg), FXIII, Mac-1, $\alpha_V\beta_3$, and more (see figure). For instance, tPA's catalytic efficiency for plasminogen is significantly enhanced 100- to 1000-fold in the presence of fibrin,[4] and patients with congenital afibrinogenemia exhibit impaired FXIII activation.[5] In view of the pivotal roles of fibrin in these processes, it is intriguing that Fga^{EK} mice exhibit normal FXIII activation, fibrinolysis, and wound-healing responses.

One plausible explanation is the functional FpB present in nonpolymerizable Fga^{EK}, which can facilitate a conformational switch when cleaved by thrombin. FpB is known to interact with the αC-domain, potentially masking binding sites within the αC-domains and the βN-domain[6] (see figure). Removing FpB leads to the dissociation of the intramolecular αC-domain:αC-domain complex, thereby exposing cryptic sites within these domains for tPA, Plg, FXIII, $\alpha_V\beta_3$, and others. Once released from its intramolecular interaction, the αC-domains are available to form intermolecular interactions between different fibrinogen molecules.[7] Additionally, the unmasked cryptic binding sites within the fibrin βN-domain can facilitate fibrin binding to the endothelium via VE-cadherin and VLDLR.[6] One potential exception to this model is the cryptic binding site for Mac-1 located within the γ-module. Mac-1 is known to preferentially recognize fibrin, and its interaction with fibrin plays a crucial role in the pathogenesis of several cardiovascular and neurological diseases.[8] Disrupting Mac-1 binding to fibrin, by mutating the Mac-1-recognition motif γ377-395 within the γ-module (the $Fib\gamma^{390\text{-}396A}$ mice), mitigates most fibrin-associated pathology.[8] Curiously, Fga^{EK} mice exhibit compromised antimicrobial host defense, which mirrors the defective host defense phenotype observed in $Fib\gamma^{390\text{-}396A}$ mice.[9] The similarity in their antimicrobial responses between the Fga^{EK} and $Fib\gamma^{390\text{-}396A}$ mice suggests that the Mac-1 binding site within Fga^{EK} fibrin likely remains cryptic, a notion that warrants further investigation.

In summary, this report highlights the potential of targeting fibrin polymerization, rather than the blood coagulation

Downloaded from http://ashpublications.net/blood/article-pdf/143/2/95/2180183/blood_bld-2023-022668-c-main.pdf by guest on 08 June 2024



Thrombin enzymatically converts the nonpolymerizable fibrinogen, Fga^{EK}, to fibrin, releasing the fibrinopeptide B (FpB). The fibrinopeptide A (FpA) cannot be cleaved due to mutations in the cleavage sequence (denoted by X). The removal of FpB abolishes its binding to the αC-domain as well as the intramolecular αC-domain:αC-domain interaction, thereby exposing cryptic binding sites in the αC-domains and the βN-domain (in the E region). Fga^{EK} fibrin(ogen) can form multimeric complexes via the "B"-knob:hole "b" interaction and intermolecular αC-domain:αC-domain interaction. VE: vascular endothelial; VLDLR, very low-density lipoprotein receptor.

cascade or platelet function, as a means to strike a delicate balance between thrombosis prevention and hemostasis maintenance. It also showcases the feasibility of harnessing the unique properties of nonpolymerizable Fga^{EK} as a promising antithrombotic agent. These include its poor capability to form a dense fibrin network yet its retention of other functionalities to support platelet aggregation, endothelial cell binding,[2,6] interactions with various protein and nonprotein ligands, and facilitation of fibrinolysis. Fortuitously, the Mac-1 binding site within this nonpolymerizable fibrinogen appears to remain concealed even after thrombin-mediated activation, potentially reducing its pathological activity in various diseases.

Previous research has explored strategies targeting fibrin polymerization, such as the use of knob-mimic peptides (GPRP and GHRP) to suppress fibrin polymerization. However, their low affinity and short circulating half-life, combined with high concentrations of fibrinogen in circulation, renders them unsuitable as antithrombotic drugs. Compared with these small peptides, Fga^{EK} could be more efficient at suppressing fibrin polymerization, as it can cap the growing end of fibrin polymer, thereby terminating the polymerization process. Indeed, $Fga^{WT/EK}$ mice, which carry both normal (WT) and nonpolymerizable (Fga^{EK}) fibrinogen, experience a significant reduction in thrombosis incidence and thrombus size while retaining normal hemostasis. Furthermore, human patients with dysfibrinogenemia (fibrinogen Detroit, Metz, Frankfurt XIII, etc), all of which harbor a mutation at the thrombin cleavage site Aα(16)Arg, are either asymptomatic or only suffer mild bleeding.[10] These clinical insights suggest that nonpolymerizable fibrinogen can be used safely in human patients to mitigate thrombotic tendencies.

It will undoubtedly be a long road to translate nonpolymerizable fibrinogen into a safe and efficient antithrombotic agent. Nonetheless, this work offers a glimmer of hope that the elusive balance between preventing thrombosis and maintaining hemostasis may be attainable in the future.

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

REFERENCES

1. Hur WS, Kawano T, Mwiza JMN, et al. Mice expressing nonpolymerizable fibrinogen have reduced arterial and venous thrombosis with preserved hemostasis. *Blood.* 2024;143(2): 105-117.

2. Weisel JW, Litvinov RI. Mechanisms of fibrin polymerization and clinical implications. *Blood.* 2013;121(10):1712-1719.

3. Drew AF, Liu H, Davidson JM, Daugherty CC, Degen JL. Wound-healing defects in mice lacking fibrinogen. *Blood.* 2001;97(12): 3691-3698.

4. Hudson NE. Biophysical Mechanisms Mediating Fibrin Fiber Lysis. *Biomed Res Int.* 2017;2017:2748340.

5. Bridey F, Négrier C, Duval C, Ariëns R, de Moerloose P, Casini A. Impaired factor XIII activation in patients with congenital afibrinogenemia. *Haematologica.* 2019; 104(3):e111-e113.

Downloaded from http://ashpublications.net/blood/article-pdf/143/2/95/2180183/blood_bld-2023-022668-c-main.pdf by guest on 08 June 2024

6. Yakovlev S, Strickland DK, Medved L. Current view on the molecular mechanisms underlying fibrin(ogen)-dependent inflammation. *Thromb Haemost*. 2022;122(11):1858-1868.

7. McPherson HR, Duval C, Baker SR, et al. Fibrinogen αc-subregions critically contribute blood clot fibre growth, mechanical stability, and resistance to fibrinolysis. *Elife*. 2021;10:e68761.

8. Petersen MA, Ryu JK, Akassoglou K. Fibrinogen in neurological diseases: mechanisms, imaging and therapeutics. *Nat Rev Neurosci*. 2018;19(5):283-301.

9. Prasad JM, Gorkun OV, Raghu H, et al. Mice expressing a mutant form of fibrinogen that cannot support fibrin formation exhibit compromised antimicrobial host defense. *Blood*. 2015;126(17):2047-2058.

10. Li Y, Ding B, Wang X, Ding Q. Congenital (hypo-)dysfibrinogenemia and bleeding: a systematic literature review. *Thromb Res*. 2022;217:36-47.

https://doi.org/10.1182/blood.2023022668

© 2024 American Society of Hematology. Published by Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

CLINICAL TRIALS AND OBSERVATIONS

Comment on Ghorashian et al, page 118

Is immune escape in the rearview mirror?

Swati Naik and Stephen Gottschalk | St. Jude Children's Research Hospital

In this issue of *Blood*, Ghorashian et al report results from a phase 1 clinical trial, in which they investigated a novel CD19/CD22-bispecific chimeric antigen receptor (CAR) T-cell product in a cohort of 12 patients with relapsed/refractory (r/r) acute lymphoblastic leukemia (ALL), and they report no antigen-negative relapses.[1]

CD19-CAR T-cell therapy has transformed the treatment of pediatric patients with r/r ALL. However, despite the impressive initial remission rates of >80% across multiple studies using Food and Drug Administration–approved or investigational CD19-CAR T-cell products, 36% to 57% of patients who achieve complete remission (CR) relapse within 1 year, and durable remission occurs in only approximately 40% to 50% of patients.[2] Outcomes post-CAR T-cell relapse remain dismal, and relapse due to emergence of $CD19^-$ leukemia, in particular, is associated with extremely poor prognosis.[3] Indeed, the emergence of $CD19^-$ disease and limited CAR T-cell persistence are the major limitations of current CD19-CAR T-cell approaches.

The incidence of $CD19^-$ ALL relapse post-CD19-CAR T cell varies and has been reported to be up to 57%.[4] Several mechanisms for this immune escape have been described including (1) mutations in CD19 that lead to shedding of the extracellular domain, (2) selection of a preexisting $CD19^-$ clone, and (3) lineage switch with the recurring leukemia having an AML phenotype.[5,6] The logical approach to overcome immune escape is to target additional antigens. This is an area of active research, and most efforts are focused on targeting CD22 based on the encouraging results of CD22-CAR T-cell therapy for pediatric ALL.

The most commonly employed CD19/22 dual targeting strategies can be summarized under 3 broad approaches: (1) sequential or coadministration of CD19 and CD22 CAR T-cell products, (2) engineering T cells to express CD19-CAR and CD22-CAR using either a single (bicistronic) vector or double (cotransduction) vectors, or (3) engineering T cells to express a single CAR that recognizes both CD19 and CD22 (bispecific).

All 3 approaches have been evaluated in early phase clinical studies with the largest cohort of patients having received 2 CAR T-cell products.[7-9] The results of these studies indicate that all 3 approaches are safe. However, relapse rates in most of these studies are >40%, and it remains to be determined which approach is best. Of note, none of the studies targeting CD19/22 have resulted in improved outcomes compared with CD19-CAR T-cell therapy alone, and none, except for the current study, have eliminated antigen-negative relapses.

This is what makes the study by Ghorashian et al unique. It is based on a strategy that was thoughtfully designed, taking into account the limitations associated with targeting CD19 and CD22 in previous studies. The CD19-targeting component utilized a CD19-CAR (AUTO1) with a fast-off rate post CD19 binding, which endows CAR T cells with enhanced effector function and showed efficacy in a previous trial.[10] To target CD22, the investigators designed a novel construct. CD22 is known to have low antigen density on ALL blasts, and relapses post CD22-CAR T-cell therapy are associated with further CD22 downregulation. Therefore, the group designed a highly sensitive CD22-CAR that recognized target cells expressing low levels of CD22.

These CD19- and CD22-CAR constructs were used to generate CD19/CD22-CAR T-cell products by cotransducing T cells with separate lentiviral vectors encoding the CD19- or CD22-CAR. The median transduction efficiency was 83.2% (range 60.8%-92.6%) with the majority of T cells expressing CD19- and CD22-CARs and having a predominant central memory T-cell phenotype. There was excellent early expansion of both CD19- and CD22-CAR T cells with high cumulative CAR T-cell exposure post infusion and also balanced engraftment of mono- and bispecific CAR T cells. This is in contrast to previous reports using CD19/22-directed CAR T-cell strategies where skewing of engrafted CAR T-cell populations was observed.

Twelve patients with advanced high-risk B-ALL who were heavily pretreated and had high-risk features often associated with risk of $CD19^-$ relapse ($CD19^-$ disease, previous receipt of blinatumomab or CD19-CAR T-cell therapy) were infused. Ten of 12 patients achieved measurable residual disease–negative CR at 2 months post infusion including 2 of 3 patients who had had $CD19^-$ disease. Of the 10 responding patients, 5 relapsed with $CD19^+$ and $CD22^+$ disease. Relapses were associated with loss of CAR T-cell persistence in 4 of the 5 relapsing patients. It is likely that the additional targeting through the highly sensitive CD22 construct, which could kill $CD19^-$ leukemia cell lines in

Downloaded from http://ashpublications.net/blood/article-pdf/143/2/95/2180183/blood_bld-2023-022668-c-main.pdf by guest on 08 June 2024