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Defibrotide:

A Swiss Army Knife Intervention in the Battle Against Cerebral Malaria

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Cerebral malaria (CM) is a severe manifestation of *Plasmodium falciparum* infection, characterized by seizures and coma, respiratory distress, hypoglycemia, and acidosis. Intensely studied for decades, important advances in understanding this clinically complex syndrome have been achieved, but highly efficacious treatment remains elusive.[1] Even with rapid delivery of antimalarial drugs on diagnosis and the best supportive care, many CM victims do not survive.[1] An effective malaria vaccine to prevent CM and other severe malaria syndromes is not yet available, making continued efforts to identify novel therapies, particularly those that can serve as adjuncts to antimalarial drugs, essential.[2] Defibrotide (DF), a mixture of single-stranded ≈50-mer DNA aptamers with a minor component of double stranded DNA that is derived from depolymerized mammalian genomic DNA,[3–5] is an exciting potential new recruit to the ranks of such adjunctive treatments. This multipotent drug displays endothelial-protective, antiischemic, anti-inflammatory, and mild anticoagulant effects and has been successfully used to treat comatose children experiencing veno-occlusive disease.[3–5]

CM develops when mature intraerythrocytic stages of *P. falciparum* adhere to the brain microvasculature, interrupt normal blood flow, and promote endothelial activation, culminating in disruption of normal vascular function[1] (Figure). Intense systemic and local inflammatory responses are believed to be important players in disease pathogenesis,[6] and more recently, a role for dysregulated hemostasis has also been suggested.[7,8] Francischetti et al identified a potential role for tissue factor (TF) in CM[9] and argued that the TF-mediated coagulation-inflammation cycle underlies the pathogenesis of this disease.[7,8] In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Francischetti et al[10] advance our understanding of malarial parasite/host interactions in this context. They argue that effective treatment of CM will require control of multiple pathological targets and propose that DF

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may be a suitable candidate (Figure). In their article, Francischetti et al show that DF at a concentration achievable in plasma blocks induction of endothelial TF-mediated coagulation by parasitized red blood cells and suppresses elastase and cathepsin G activity.[10] The latter observation is intriguing because cathepsin G induces platelet aggregation[3–5] and can cleave factor X, thereby promoting coagulation.[11] Elastase decreases TF pathway inhibitor levels[12] and cleaves membrane-bound thrombomodulin.[13] This is notable because brain thrombomodulin expression is already among the lowest within human tissues.[6,14] Maintenance of thrombomodulin by DF with simultaneous suppression of TF-induced coagulation and platelet aggregation could therefore provide protection against malaria-induced coagulopathy in the brain. Prompted by the recent identification of dendritic cells (DCs) as prominent in the coagulation-inflammation cycle,[15] Francischetti et al further show that at a concentration achievable in vivo, DF suppresses Toll-like receptors 4 and 2 agonist-driven proinflammatory cytokine production by DCs.[10] Toll-like receptor 2 suppression is particularly noteworthy in this context because *P. falciparum*-derived glycosylphosphatidylinositols (*Pf*GPIs) interact with this receptor,[16] and Francischetti et al demonstrate that *Pf*GPIs promote DC activation and inflammatory cytokine production.[10] Moreover, *Pf*GPIs initiate TF-mediated procoagulant activity on microvascular endothelial cells in a cytokine-independent manner. At drug concentrations higher than might be found in vivo, Francischetti et al further show that DF directly decreases platelet aggregation and blocks the alternative complement pathway.[10] The latter is noteworthy because parasitized red blood cells enhance complement component 5 activation, and *Pf*GPIs promote expression of the complement component 5a receptor on human monocytes. Together, complement component 5a and *Pf*GPI induce inflammatory cytokine and chemokine production and may suppress angiogenesis,[17] dysregulation of which is observed in CM.[18] Thus, in both DCs and endothelial cells, DF suppresses inflammatory cytokine secretion, promotes production of anti-inflammatory factors, blocks TF-mediated coagulation, and supports inhibition of coagulation. Although the mechanistic basis for these effects remains to be established, Francischetti et al[10] consider that DF may act through adenosine receptors.[19] Consistent with this, they observe that DF-exposed DCs produce prostaglandin E_2 and have enhanced lipopolysaccharide-induced IL-10 secretion.

In addition to a potential for interrupting the coagulation-inflammation cycle, DF may act directly on the malarial parasite. Francischetti et al show that DF blocks parasite invasion of red blood cells and rosetting, the agglutination of parasitized red blood cells with other infected and uninfected red blood cells.[10] Furthermore, inclusion of the drug in parasitized blood fed to mosquitoes decreases midgut parasite development. Finally, in a murine model for CM, treatment with DF thrice daily starting at day 1 of infection delays development of parasitemia and neurological signs and significantly reduces systemic interferon-γ, which is important in disease pathogenesis in this model[20,21]; there is also a trend toward extended time to death. Initiation of treatment after development of significant parasitemia (day 4) does not have these effects, suggesting that it may be less effective in controlling malaria-induced inflammation and coagulation once established. Although these in vivo data show modest drug efficacy, considered together with the in vitro work, these observations at minimum provide leads for critical future research (Figure). For example, detailed characterization of the role of adenosine receptors in the DF response in the context of

malaria could help to optimize drug efficacy and identify specific components that are most stable in the face of degradative plasma exonucleases.[3–5] Additionally, the impact of DF on protease-activated receptor activity should be explored. On cleavage by coagulation proteases, protease-activated receptors promote inflammatory responses and endothelial cell activation,[22] and, indeed, are pivotal for the participation of DCs in the coagulation-inflammation cycle. To date, the role of protease-activated receptors has not been investigated in malaria, but they are clearly involved in the pathophysiology of sepsis and several other disease conditions.[22] Moreover, there is intriguing evidence that protease-activated receptor and adenosine receptor signaling are coupled.[23]

In summary, Francischetti et al not only reemphasize the importance of the coagulation-inflammation cycle in the pathogenesis of CM but also introduce a potential new therapeutic approach for treatment of this deadly disease. Importantly, DF is a safe and efficacious treatment already in clinical use.[3–5] Given its multipotent affects, DF or related compounds used in conjunction with effective antimalarial drugs could revolutionize clinical management of CM. It also deserves mention that CM patients may not be the only beneficiaries of new treatment approaches using DF/antimalarial drug combinations. Recent evidence suggests that placental malaria pathogenesis is also driven by the coagulation-inflammation cycle,[24,32] making malaria-exposed pregnant women another group who might be considered for DF-containing adjunctive treatments.

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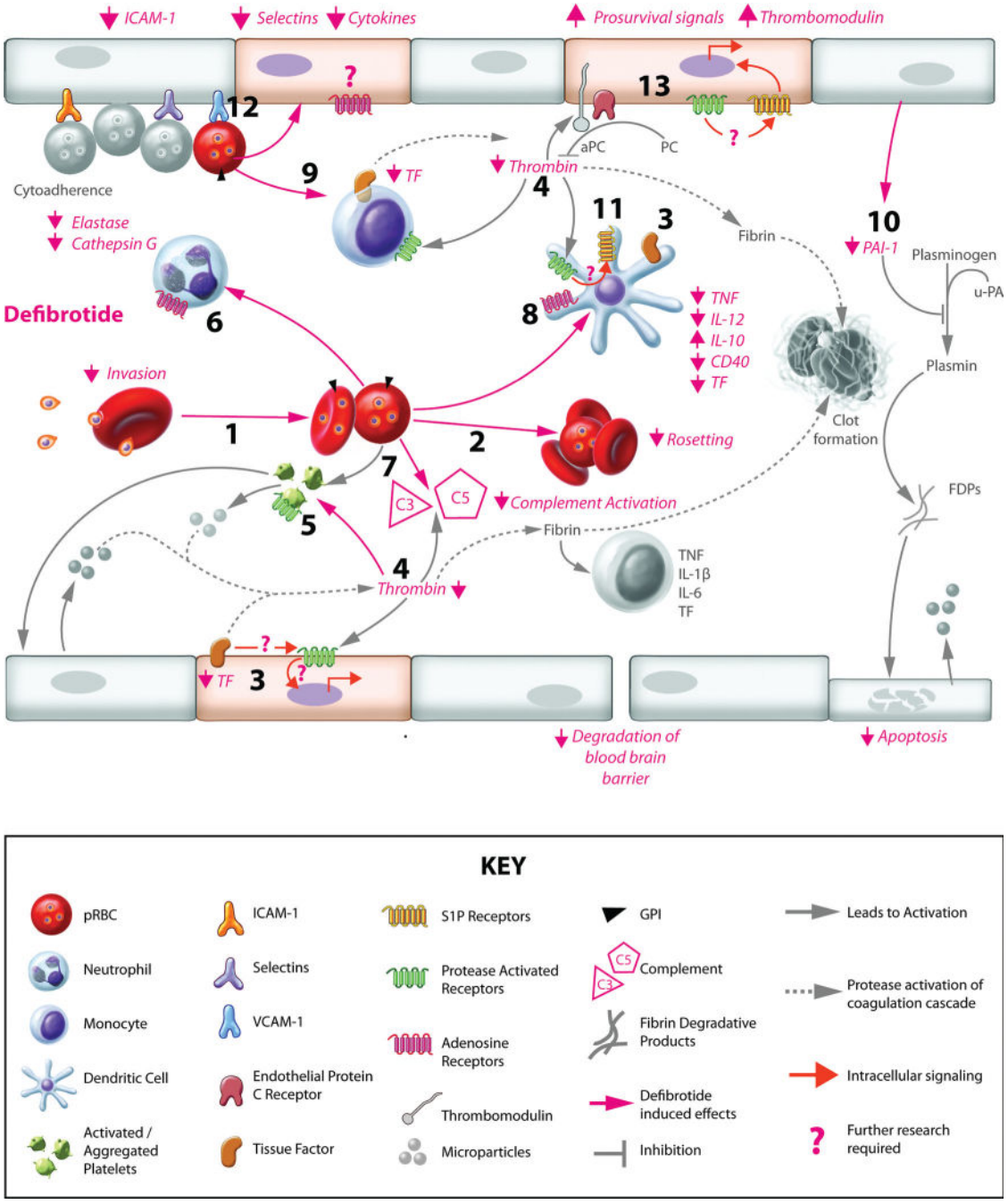

Figure.

Defibrotide (DF) and the coagulation-inflammation cycle in cerebral malaria (CM). CM-induced dysregulation of coagulation and inflammation is precipitated by adherence of *Plasmodium falciparum*–infected red blood cells (pRBCs) to endothelial cells (ECs), pRBC/red blood cell (RBC) rosettes, inflammatory infiltrate and cytokine production, and fibrin clots, leading to occlusion of the brain microvasculature and degradation of the blood-brain barrier. As shown by Francischetti et al,[10] DF may protect against CM by acting directly on *P. falciparum*. Specifically, DF (1) inhibits RBC reinvasion by merozoites and

(2) decreases rosette formation. Additional DF benefits may be suppression of coagulation through (3) reduced tissue factor (TF) expression and activity, (4) reduced activation of thrombin, (5) decreased platelet activation and aggregation,[25] and (6) diminished activity of neutrophil-derived elastase and cathepsin G. Also relevant to CM inflammatory pathogenesis, DF (7) suppresses complement activation and (8) modulates dendritic cell (DC) activation through adenosine receptors (ARs), decreasing CD40, tumor necrosis factor (TNF), and interleukin (IL)-12 expression and increasing immunoregulatory IL-10 expression. Other previously reported important effects of DF with relevance to CM include (9) reduced activation and downregulated proinflammatory/procoagulant status in monocytes[26]; (10) decreased plasminogen activator inhibitor (PAI)-1 and (3) TF expression, which reduces fibrin clot maintenance, coagulation initiation, and TF/protease-activated receptor (PAR) signaling,[5,27] potentially involving (11) sphingosine-1 phosphate (S1P) receptors on DCs[15]; (12) decreased leukocyte and possibly pRBC recruitment by reducing the expression of EC adherence molecules,[28] including intercellular adhesion molecule (ICAM)-1; (13) and increased expression of thrombomodulin[29] and stabilization of ECs.[30] u-PA indicates urokinase-type plasminogen activator; FDPs, fibrin degradation products; GPI, glycosylphosphatidylinositol; VCAM, vascular cell adhesion molecule. Areas that require further study (“?”) include the role of PARs in CM and the extent to which ARs on ECs and DCs are critical in the DF-induced protective response against CM. Paradoxically, S1P receptors are implicated in the coagulation-inflammation cycle,[15] yet availability of S1P is important for protection against CM;[31], therefore, the interactions between ARs, PARs, and S1P receptors, both in pathogenesis and in the context of DF treatment, should be investigated.