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The KLF4-CD46 axis: a novel therapeutic target in transplant-associated thrombotic microangiopathy and beyond

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In this issue of Haematologica, Jiang et al evaluated the role of the transcription factor Krüppel-like factor 4 (KLF4) in the pathophysiology of transplant-associated thrombotic microangiopathy (TA-TMA) by combining *ex vivo* data from 20 TA-TMA patients and experimental data in cellular and animal models [1].

TA-TMA is a severe and often life-threatening complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Despite the many efforts made to understand the mechanisms of TA-TMA, its pathogenesis is largely unknown, the case-fatality rate remains high and survivors experience chronic consequences such as kidney disease and neurological impairment. It is believed that complement hyperactivation and other proinflammatory and procoagulant factors cause endothelial injury resulting in microvascular thrombosis and tissue damage. A three-hit model is widely accepted: pre-existing endothelial injury or underlying complement activation predisposition (hit 1), endothelial toxicity from the conditioning regimen (hit 2), and post-transplant injuries caused by medications, alloreactivity, and/or infections (hit 3) [2]. Even though therapies targeting complement activation, such as eculizumab [3] and narsoplimab [4], have been used and other new drugs are undergoing clinical evaluation [5], outcomes remain suboptimal. Thus, there is a need for novel interventions to restrain complement activation and to preserve endothelial integrity. Clinically available biomarkers of complement activation [6] could be useful for tailoring the therapies and assessing their efficacy.

KLF4 is a transcription factor regulating several cellular processes such as cell growth, proliferation, and differentiation. In particular, KLF4 suppresses the activation of inflammatory signaling by inducing the expression of multiple anti-inflammatory and anti-thrombotic factors [7]. In the context of TA-TMA, Jiang et al [1] show that KLF4 stimulates the expression of CD46 (cluster of differentiation 46), also known as membrane cofactor protein (MCP). MCP is a cofactor for the proteolytic inactivation of complement components C3b and C4b by complement factor I

(CFI), protecting host cells from damage by complement [8]. The role of the KLF4-CD46 axis as a mechanism protecting from TA-TMA is a novel aspect that might have relevance in the pathophysiology of this complication.

Thrombosis in the microcirculation, responsible for the clinical features of TA-TMA, is associated with platelet consumption, microangiopathic hemolysis and organ dysfunction, especially involving the kidney. Nevertheless, the diagnosis remains very difficult because TA-TMA occurs early after allo-HSCT, at a time when cytopenia, including low platelet count, may be related to still insufficient marrow function or to coexisting complications like infections and/or immune-mediated injury and/or drug toxicity. In the majority of cases, the risk of bleeding prevents histological evaluation, which would be the gold standard of TA-TMA diagnosis. Thus, several attempts to refine and standardize the definition of TA-TMA have been made in the past few years and a consensus proposal to harmonize the diagnostic criteria has been recently made by the world's leading blood and marrow transplant societies [9]. This consensus defines that TA-TMA is diagnosed when at least four of the following seven features are present at least twice over a 14-day window: anemia, thrombocytopenia, hypertension, increased LDH, schistocytes, elevated soluble C5b-9 and proteinuria [9]. Jiang et al applied these criteria [1] and found 20 patients with TA-TMA out of 2387 allo-HSCT recipients who were analyzed retrospectively. A certain degree of uncertainty still remains because of the overlap of TA-TMA with other clinical conditions showing similar alterations, such as graft versus host disease (GVHD), infections and drug toxicity. Despite these potential limitations, the authors found increased levels of markers of complement activation and of endothelial injury in plasma of their TA-TMA patients. In contrast, they documented reduced plasma levels of circulating KLF4. Incubating cultures of human umbilical vein endothelial cells (HUVECs) with plasma of TA-TMA patients, proinflammatory and prothrombotic endothelial markers as well as the cell deposition of activated complement components were studied in adherent (intact) HUVECs and detached endothelial cells (surrogate for circulating endothelial

cells). KLF4 transcripts and protein were markedly down-regulated in detached endothelial cells as compared to intact HUVECs, suggesting that diminished KLF4 expression compromised endothelial resistance. Treatment of HUVECs with the KLF4 stimulator APTO253 as well as the lentiviral vector-mediated overexpression of KLF4 restored these alterations. To clarify the mechanisms of KLF4-mediated endothelial protection the authors used an enzyme-tethering strategy that provides efficient high-resolution sequencing libraries for profiling diverse chromatin components, i.e. CUT&Tag (cleavage under targets and tagmentation) [10]. Using CUT&Tag, RNA sequencing, transfection and luciferase assays, Jiang et al concluded that KLF4 upregulates CD46 expression in HUVECs [1]. Finally, they used an animal model of Dimethyloxalylglycine (DMOG)-treated mice; such mice show a phenotype resembling that seen in TA-TMA patients. Treatment with the KLF4 stimulator APTO253 reduced hemolysis, increased platelet number and reduced markers of endothelial damage. APTO253 also improved kidney lesions, as shown by histological analysis, and decreased C3/C5b-9 deposition, as shown by immunofluorescence. Quantitative real-time PCR and Western blot analyses confirmed KLF4 overexpression in renal tissues. Using the same methodology a similar improvement was observed in mice treated with pravastatin, which was administered via oral tube at the dosage of 20 mg/kg daily.

If the data of Jiang et al [1] are confirmed, KLF4-CD46 axis may have an important place in the pathophysiology of TA-TMA (Figure 1). Indeed, endothelial injury and complement activation are considered the central aspects of TA-TMA pathophysiology (for rev. see [2]). Several factors in the transplantation process, such as chemotherapeutic agents, conditioning regimens, infections, and graft-versus-host disease (GVHD), lead to endothelial injury and to an increase in proinflammatory cytokines, prothrombotic factors and complement activation, which in turn further damage the endothelium. Patients with variants of complement system regulatory proteins predisposing to complement hyperactivation, also acquired from their HSC donors, appear to be at increased risk for TA-TMA [11,12]. Neutrophil extracellular traps (NETs) may also link endothelial injury and

complement activation [13]. The reduced plasma levels of KLF4 leading to low expression of CD46 render the endothelium more vulnerable to complement activation in TA-TMA. The possibility of pharmacologically increasing KLF4 levels with the subsequent increase of endothelial CD46 will protect against complement hyperactivation and TMA. The stimulating effect of pravastatin on KLF4, may defend the endothelium also thanks to the statin-mediated and KLF4-dependent up-regulation of atheroprotective and antithrombotic genes, as nitric oxide synthase 3 (NOS3) and thrombomodulin (THBD), respectively [14]. Beyond TA-TMA, the therapeutic benefits could be extended to other syndromes characterized by complement-driven endothelial injury, such as atypical hemolytic uremic syndrome (aHUS) and possibly antiphospholipid syndrome (APS). In these conditions, as well as in all vascular complications of allo-HSCT, the KLF4-CD46 pathway may provide a generalizable mechanism for restoring vascular homeostasis in concert with the other previously demonstrated anti-inflammatory and antithrombotic effects of KLF4 [15].

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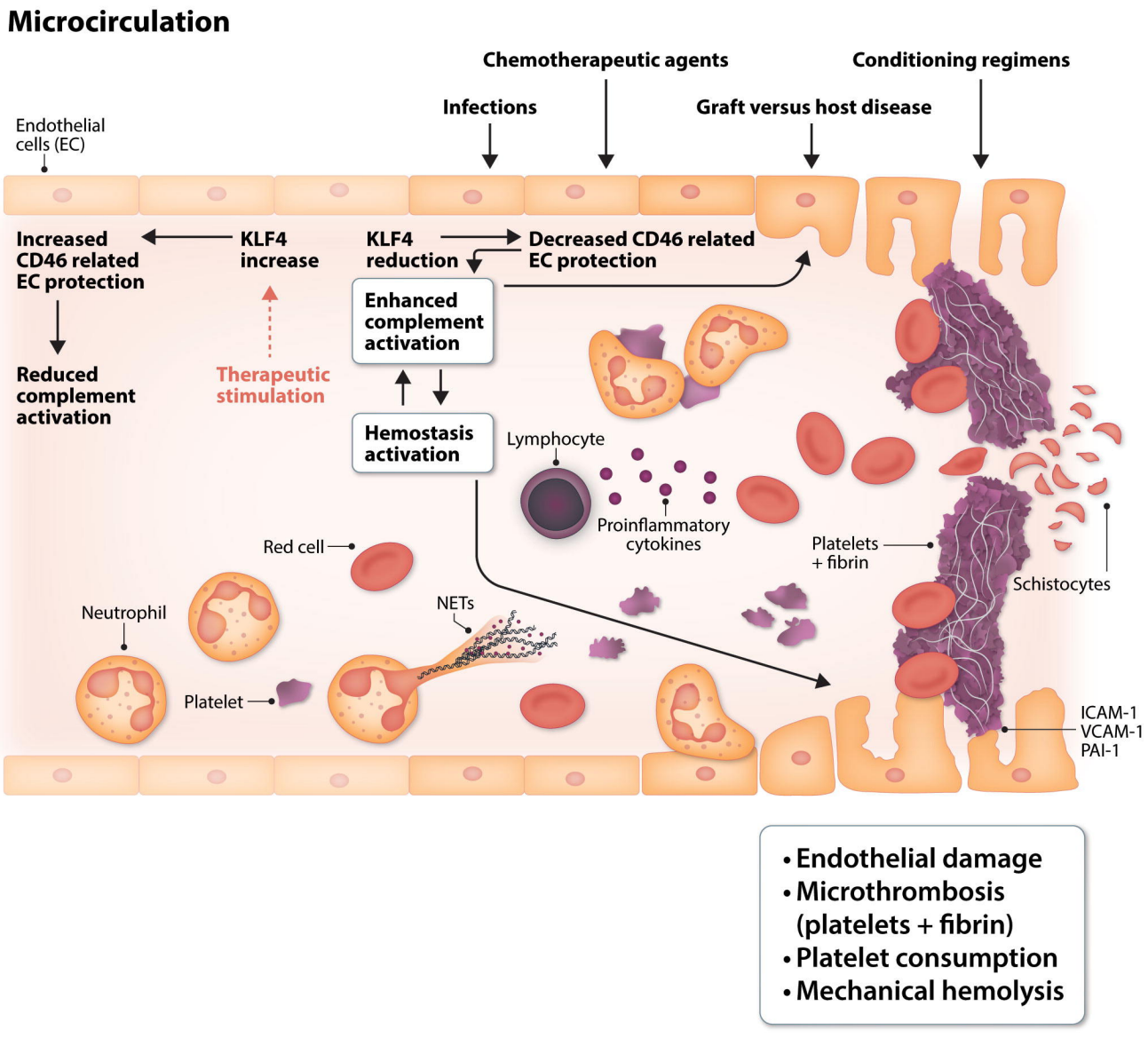
FIGURE LEGEND

Figure 1. Pathophysiology of transplant-associated thrombotic microangiopathy and possible therapeutic intervention. Infections, chemotherapeutic agents, graft-versus-host disease and conditioning regimens lead to endothelial injury and to an increase in proinflammatory cytokines, prothrombotic factors and complement activation, which in turn further damage the endothelium. A predisposition to complement hyperactivation may be due to the presence of variants of complement system regulatory proteins. Neutrophil extracellular traps (NETs) link endothelial injury and complement activation. The reduced plasma levels of KLF4 leading to low expression of CD46 (complement inhibitor also known as membrane cofactor protein [MCP]) render the endothelium more vulnerable to complement activation. Therapeutically increased KLF4 levels may induce an increase of endothelial CD46 and thus reduce complement activation and protect endothelial cells.


Microcirculation
Chemotherapeutic agents
Conditioning regimens
Infections
Graft versus host disease
Endothelial cells (EC)
Increased CD46 related EC protection
KLF4 increase
KLF4 reduction
Decreased CD46 related EC protection
Reduced complement activation
Therapeutic stimulation
Enhanced complement activation
Hemostasis activation
Lymphocyte
Proinflammatory cytokines
Red cell
Neutrophil
NETs
Platelet
Platelets + fibrin
Schistocytes
ICAM-1
VCAM-1
PAI-1
• Endothelial damage
• Microthrombosis (platelets + fibrin)
• Platelet consumption
• Mechanical hemolysis