

Comment on Shimazu et al, page 59

# Plasmin regulation of acute cytokine storm

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In this issue of *Blood*, Shimazu et al report that cytokine release and activation is mediated through a previously unrecognized TLR9/plasmin/MMP9 axis.<sup>1</sup>

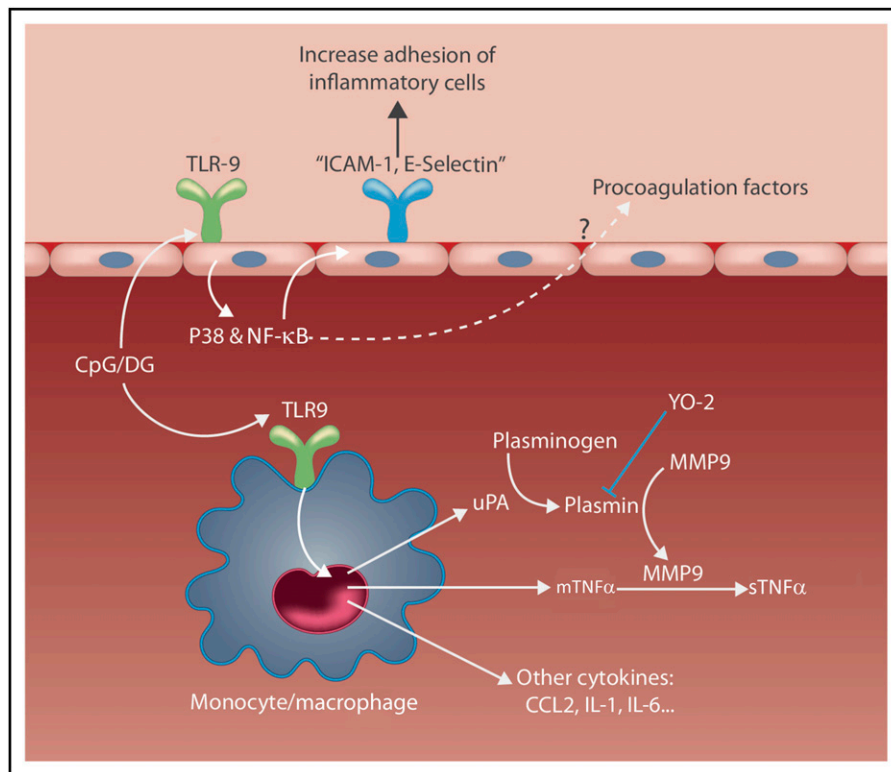
**M**acrophage activation syndrome (MAS) is a life-threatening complication associated with systemic juvenile idiopathic arthritis or with adult-onset Still disease.<sup>2</sup> This syndrome has several attributes common to hemophagocytic lymphohistiocytosis (HLH), because both of these life-threatening conditions are characterized by a

supraphysiological elevation of cytokines, an increase in inflammation, multiorgan dysfunction, and death.<sup>2</sup> Although MAS is generally treated with corticosteroids, this treatment is not effective in all patients. Refractory patients have been treated with prototypical immune modulators, including cyclosporine A, tumor necrosis factor (TNF)

inhibitors, or anti-interleukin-1 or -6 therapies,<sup>2</sup> with marginal benefit. In search of the pathogenesis of MAS and HLH, Shimazu et al studied the function of plasmin activation in response to an acute cytokine storm that is the basis of and driver for many of these diseases. Their results demonstrate how plasmin activation contributes to the regulation of the inflammatory response through the activation of TNF $\alpha$  among other cytokines.

To establish an in vivo model of MAS, cytosine guanine (CpG) bacterial DNA was injected into mice.<sup>3</sup> However, this model developed a mild inflammatory response associated with the activation of Toll-like receptor 9 (TLR-9) by CpG that did not reproduce the exacerbated inflammation observed in patients with MAS. This can be achieved by the coinjection of CpG with D-galactosamine (DG) to induce lethal hepatitis in mice through a dramatic increase in TLR-9-mediated TNF $\alpha$  activation, as has been previously described.<sup>4</sup> Using this model system of CpG/DG coinjection, Shimazu et al not only were able to phenocopy the onset of MAS syndrome but also have uncovered the role of plasmin in the dysregulation of inflammation and coagulation that leads to MAS.

Plasmin is the active form of the enzyme plasminogen and is involved in the processing and degradation of many blood plasma proteins, including fibrin clots. Although the role of plasmin in inflammation has been previously described,<sup>5</sup> its role in the development of inflammatory syndromes is undefined. Therefore, the use of plasmin-deficient mice has been useful in the characterization of its role in mediating inflammatory reactions. However, a major disadvantage of plasmin-deficient mice is the development of diverse pathologies associated with fibrin deposition.<sup>6</sup> The chemical inhibition of plasmin in wild-type mice offers an alternative model. Notably, the authors observed that the chemical inhibition of the active center of plasmin with trans-4-aminomethylcyclohexanecarbonyl-Tyr(O-Pic)-octylamide (YO-2), after CpG/DG administration, was more effective when administered at early stages of inflammation, decreasing the inflammatory markers in blood and inflammatory cells in the liver, spleen, and bone marrow, leading to an increase in survival. This finding suggests that plasmin could contribute to the rapid onset of inflammatory response, although further studies should



Plasmin has a central role in the regulation of the inflammatory response via MMP9 activation. Administration of CpG/DG activates TLR-9 in different cell populations, which stimulates the secretion of urokinase-type plasminogen activator that will modify plasminogen into its active form, plasmin. The activation of TLR-9 in monocyte/macrophages will activate production of a cytokine storm, which involves the production of transmembrane TNF $\alpha$ , chemokine ligand 2 (CCL2), and interleukin-1 (IL-1) and IL-6. Notably, the generation of plasmin promotes the activation of MMP9, which will cleave mTNF $\alpha$  into its soluble form (sTNF $\alpha$ ), increasing the inflammatory response. The transient inhibition of plasmin with YO-2 decreases the inflammatory response, increasing the viability of treated mice; however, it did not affect the increase in blood coagulation markers, suggesting that CpG/DG activates the coagulation cascade in a plasmin-independent manner. This response might be mediated by the activation in endothelial cells of the NF $\kappa$ B pathway, which has been described as being activated by CpG and being involved in the inflammatory and coagulation responses mediated by endothelial cells. mTNF $\alpha$ , membrane TNF- $\alpha$ ; uPA, urokinase-type plasminogen activator. Professional illustration by Somersault18:24.

determine its contribution to chronic inflammatory conditions.

Although pharmacological inhibition of plasmin controlled acute inflammation and liver damage, it did not diminish the activation of the coagulation pathway. Although these results could be due to a different pathway involving fibrin deposition and fibrin-associated inflammatory response as suggested by the authors, the administration of CpG/DG also activates a TLR-9 response in endothelial cells that triggers the activation of the NFκB pathway.<sup>7</sup> This latter pathway has been related to the activation of the coagulation cascade<sup>8</sup> (see figure). Furthermore, the genetic deletion of plasmin in mice generates spontaneous thrombosis,<sup>6</sup> thereby limiting the opportunity for therapeutic intervention. Nonetheless, despite these limitations, the global decrease of the inflammatory response and the increase in survival were significant achievements.

The authors noted that TLR-9-driven TNFα activation was mediated through a plasmin/matrix metalloproteinase 9 (MMP9) axis (see figure). They show how in MMP9-deficient mice, the activation with CpD/DG was significantly diminished, resulting in improved survival. Importantly, these data are supported by previous reports, demonstrating that MMP9 deficiency has a protective effect in response to lipopolysaccharide challenge by reducing the inflammatory response.<sup>9</sup> Moreover, similar results have been recently described using a blocking antibody against TLR-9 after the administration of CpG/DG,<sup>10</sup> confirming the importance of the TLR-9/plasmin/MMP9 axis in the control of the cytokine storm response.

In summary, the article by Shimazu et al describes a relevant model of MAS in which TLR-9 stimulation contributes to the activation of plasmin, augmenting the inflammatory response through the control of MMP9. This study reveals the importance of plasmin in the control of acute inflammatory cytokine production and opens the door for the development of alternative therapeutic approaches for treating syndromes associated with an acute cytokine storm, such as MAS or HLH.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

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## ● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Wang et al, page 73

# Hemochromatosis, iron-loading anemia, and SMAD

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In this issue of *Blood*, Wang et al show that SMAD1 and SMAD5 act cooperatively to increase hepatic hepcidin expression in response to iron-mediated bone morphogenetic protein (BMP) signaling, and they provide evidence that erythroferrone produced by bone marrow progenitors may suppress hepatic hepcidin expression by inhibiting these same factors (see figure).<sup>1</sup>

**H**ow patients with anemias characterized by ineffective erythropoiesis develop systemic iron overload in the absence of blood transfusions is a fascinating question that hematologists have pondered for many years. Thalassaemia major and intermedia syndromes are important examples of the association of the increased erythropoietic activity of intramedullary hemolysis with enhanced intestinal iron absorption.<sup>2</sup> The magnitude of iron overload that may occur in conditions marked by ineffective erythropoiesis is independent of the degree of anemia,<sup>3</sup> and the predominantly parenchymal iron loading in ineffective erythropoiesis is similar to hereditary hemochromatosis. These observations raised the possibility that the 2 conditions share in common a final pathophysiologic pathway.<sup>4</sup> Indeed, this proved to be the case; it emerged over the past 15 years that hepcidin produced by hepatocytes has a central role in iron homeostasis and that deficiency of hepcidin with respect

to the body's iron burden underlies the iron loading seen in both hereditary hemochromatosis and anemias characterized by ineffective erythropoiesis.<sup>5</sup>

*HFE*, *HJV* encoding hemojuvelin, *HAMP* encoding hepcidin, and *TFR2* encoding transferrin receptor 2 are genes of the hepcidin-activating pathway in hepatocytes; autosomal-recessive inactivation of any 1 of these genes leads to deficiency of hepcidin and systemic iron overload or hemochromatosis.<sup>5</sup> Investigation of the function of these genes showed that BMP signaling through phosphorylation of SMAD proteins is a central pathway to regulate hepcidin transcription.<sup>6,7</sup> In particular, previous research indicated that iron-related BMP signals lead to the phosphorylation of SMAD1, SMAD5, and SMAD8 and to the promotion of hepcidin transcription through the common mediator SMAD4.<sup>7,8</sup> In this issue, Wang et al explored the individual contributions of SMAD1,