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

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DOI 10.1182/blood-2016-02-697169

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Comment on Das et al, page 1666

Alleviating the storm: ruxolitinib in HLH

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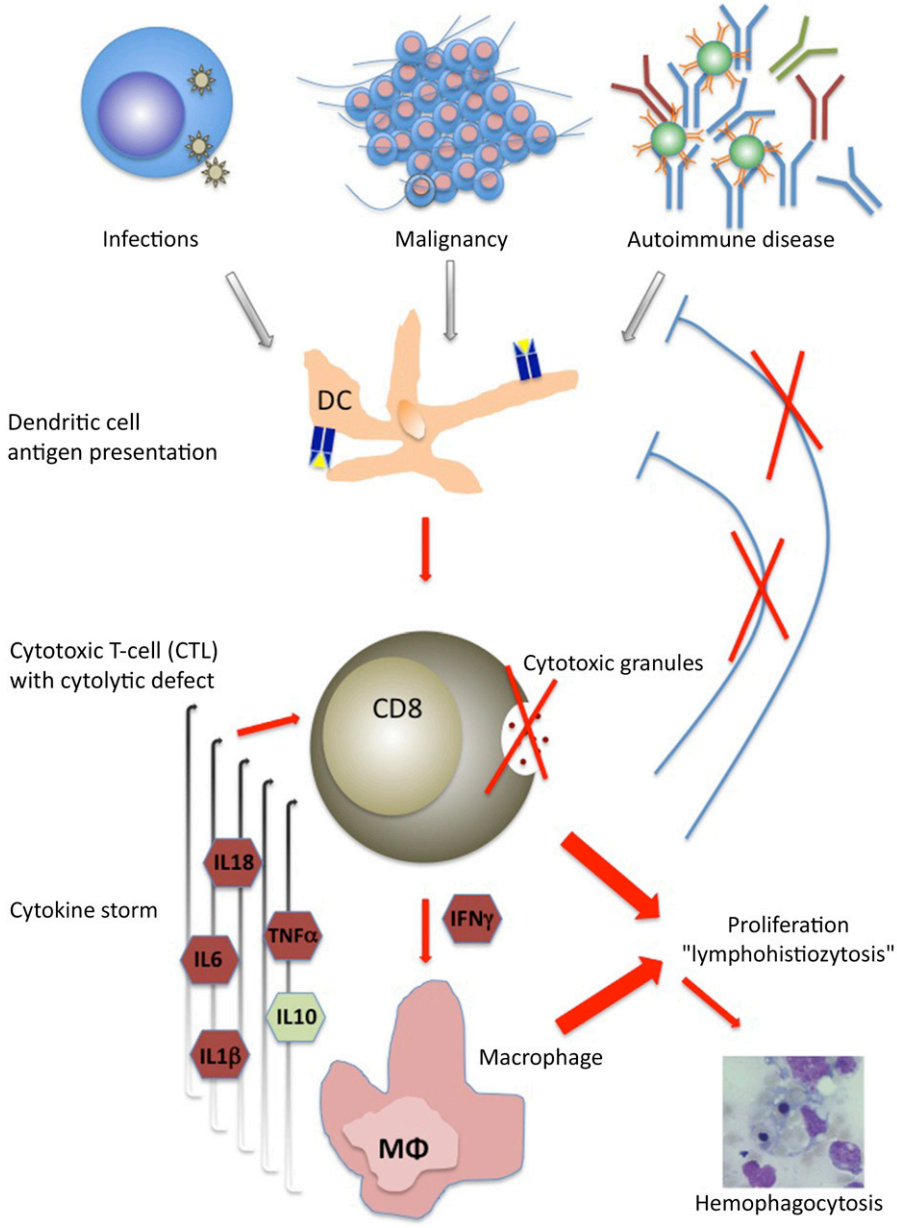
In this issue of *Blood*, Das and colleagues report their results on the use of the Janus kinase 1/2 (JAK1/2) inhibitor ruxolitinib in murine models of hemophagocytic lymphohistiocytosis (HLH), and the HLH-sibling macrophage activation syndrome (MAS).[1]

They used genetically engineered perforin-deficient *Prf1*$^{-/-}$ mice as a model of familial HLH with impaired adaptive immunity due to deficiency of cytolysis. They also used a model for acquired HLH/MAS without perforin deficiency which induces inflammation via Toll-like receptor 9 activation. Due to the central role of cytokines in HLH with JAKs critical for cytokine signaling, Das and colleagues decided to test the pharmacologic JAK1/2 inhibitor treatment as a therapeutic strategy in HLH mouse models. HLH/MAS are hyperinflammatory syndromes that share many characteristics of other highly lethal cytokine storm-associated diseases like sepsis and systemic inflammatory response syndrome. All of these disorders have unresolved issues with respect to the underlying pathophysiology, precise diagnosis, and optimal treatment.[2,3] In familial HLH, a disease usually diagnosed in newborns and toddlers, immunosuppression with high-dose corticosteroids, T-cell depletion by etoposide (VP-16), and cyclosporine A are used as a bridge to allogeneic stem cell transplantation. The HLH-1994 protocol is currently considered the therapeutic standard.[3] Despite establishing a new higher standard for HLH therapies, treatment according to the HLH-1994 protocol achieved a 5-year survival rate of 54% with a third of the patients not achieving a significant remission by induction treatment, and dying prior to hematopoietic stem cell transplantation. In adults, HLH has gained more attention, both through increased diagnostic vigilance and more patients treated with long-term immunosuppression or chemotherapy.[4] Treatment is more individualized due to a wider spectrum of underlying conditions and diseases that may trigger its onset, such as infections, cancer, and autoimmune disorders (see figure).[5] So far, a combined approach targeting both overt inflammation by immunosuppression and the underlying trigger by disease-specific treatment is rapidly initiated at diagnosis. Unfortunately, there is no sound controlled data available on efficacy and toxicity of this adult HLH treatment approach.[3] Most recently, the first prospective clinical trial of adult patients with refractory HLH was published in this journal, demonstrating that intense immunosuppression combined with chemotherapy can rescue a significant fraction of patients not responding to the HLH-1994 protocol.[6] More targeted treatment to inhibit central inflammatory cytokine pathways like interleukin 1 receptor (IL1R), IL6, or IFNγ is also being used in HLH and reported as case series or phase 1 trials.[3,7]

Ruxolitinib has demonstrated remarkable activity in other hyperinflammatory, cytokine-governed diseases. It is approved for use in myelofibrosis (MF), where it reverses the hyperinflammatory state and thereby the conditional symptoms of MF.[8] More recently, corticosteroid-resistant acute graft-versus-host disease (aGVHD), another acute syndrome of inflammation, was reported to rapidly respond to ruxolitinib after failure of standard treatment.[9,10] Ruxolitinib suppresses proinflammatory cytokines, reduces T-cell proliferation, and reverses organ damage within days through interference with JAK–signal transducer and activator of transcription (STAT) signaling. The in vivo model provided by Das and colleagues shows similar immunologic effects: the master regulator IFNγ is significantly suppressed along with TNFα. Inflammatory liver foci and T-effector cells appeared reduced. Splenomegaly and weight loss were reversed. JAK-STAT–induced gene expression in HLH mice is significantly affected as shown by reduced expression of *IFNγ*, *STAT1*, and interferon-regulating factor 1 (*IRF1*) in ruxolitinib-treated HLH mice.

A plethora of cytokine receptors use JAKs as mediators of ligand binding and initiators of the STAT-regulated gene expression programs. Mechanistically, JAK inhibition seems to be a rather promiscuous business, that is, not "precision medicine." On the other hand, the cytokine storm in HLH is also quite promiscuous: IFNγ, IL1, IL6, IL18, TNFα, and other critical proinflammatory cytokines are responsible for inflammation-driven organ damage (see figure).[5] Conversely, IL10, an



Model of HLH pathophysiology (adapted from Brisse et al[5]). Dendritic cells (DCs) present antigens of various origins, and stimulate immune cell proliferation. Due to an inherited or acquired defect in degranulation and cytolysis, negative feedback regulation is disabled leading to the vicious circle of the proliferation of $CD8^+$ T cells and macrophages producing organ damage and hemophagocytosis. A plethora of proinflammatory cytokines are involved with interferon-γ (IFNγ) being a central regulator of the subsequent cytokine storm (proinflammatory cytokines in red, anti-inflammatory interleukin 10 [IL10] in green). TNFα, tumor necrosis factor α.

anti-inflammatory mediator can also be inhibited by ruxolitinib which theoretically could induce detrimental inflammation. Moreover, cellular immune function (degranulation and cytolysis) could be negatively affected by ruxolitinib, potentially making patients even more vulnerable to complicating infections. The authors considered all of these often-conflicting effects but used their mouse model to provide compelling evidence that the rather multidirection effects of ruxolitinib on the cytokine storm are beneficial in HLH.

Can ruxolitinib become the new "one-size-fits-all treatment" for HLH? Ruxolitinib is a very potent anti-inflammatory drug, but HLH is a fatal inflammatory epiphenomenon with various underlying diseases and conditions. Ruxolitinib, which is now being evaluated in a clinical HLH trial (NCT02400463), very likely shows similar anti-inflammatory activity as demonstrated in human aGVHD. However, this does not mean that disease-specific treatment should not be given, when a specific HLH trigger can be identified.[3] Assuming ruxolitinib is effective in HLH, incorporating ruxolitinib into individualized HLH treatment to effectively tackle the cytokine storm, and to define its role in comparison with established HLH therapeutics such as etoposide, cyclosporin A, corticosteroids, or liposomal doxorubicin, will be a significant challenge. Despite many open questions, the work of Das and colleagues contributes an important piece of evidence for a novel HLH treatment that is already on its way into clinical application.

Conflict-of-interest disclosure: P.L.R. receives research support, travel support, and speakers' honorarium from Novartis Pharmaceuticals. ■

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DOI 10.1182/blood-2016-02-697151

2016 127: 1626-1627
doi:10.1182/blood-2016-02-697151

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.