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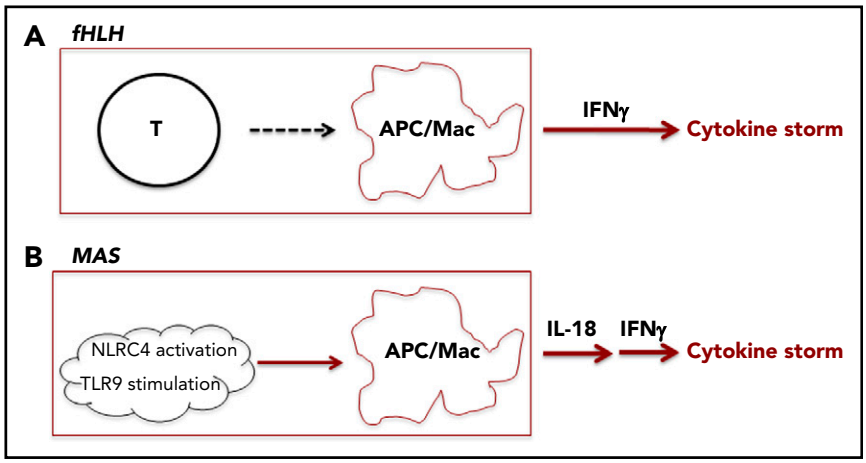
Fire behind the fury: IL-18 and MAS

Kenneth L. McClain[1,2] and Carl E. Allen[1,2] | [1]Baylor College of Medicine; [2]Texas Children's Cancer Center

In this issue of *Blood*, Weiss et al and Girard-Guyonvarc'h et al demonstrate the ability of free plasma interleukin-18 (IL-18) to distinguish macrophage activation syndrome (MAS) from other inflammatory disorders. Furthermore, with several animal models, they demonstrate that IL-18 is not just a biomarker but rather a driver of inflammation and potential therapeutic target in some patients with MAS.[1,2]

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome driven by a range of inherited and acquired factors that lead to extreme inflammation. In patients with familial HLH (fHLH) resulting from severe defects in genes regulating cytotoxic granule release (*PRF1*, *UNC13D*, *STX11*, and *STXBP2*), failure to clear antigen leads to acute hyperimmune activation with progressive organ damage and death unless inflammation is controlled with aggressive immune suppression. Some patients with autoimmune disease, notably systemic juvenile idiopathic arthritis and systemic lupus erythematosus, develop similar symptoms of fever, organ failure, and characteristic hemophagocytosis on bone marrow aspirate. In the setting of autoimmune disease (or other persistent antigenic stimulus such as cancer), HLH may be regarded as MAS. Distinguishing HLH from MAS (and HLH/MAS from severe sepsis) is not simply an academic exercise, because HLH is nearly universally fatal in infants with severe defects in cytotoxic lymphocyte function without prompt recognition and treatment.[3] By contrast, patients with MAS may respond to therapy directed against the inciting antigen(s).[4] There are no tests proven to distinguish HLH from MAS (or from severe sepsis or other mimics). Perforin-deficient mouse models suggest interferon-γ (IFN-γ) production as the critical driver of HLH, with activation of multiple downstream pathways that regulate and respond to inflammation.[5] However, the cytokine storm reflected in plasma of patients with inherited HLH and MAS has been indistinguishable (elevated CXCL9/IFN-γ, TNF-α, sIL-2Rα, IL-1, and IL-6).[6,7] Differentiating MAS from HLH is further confounded by recent observations of up to 40% of patients with MAS having monoallelic mutations in the common HLH-associated cytotoxicity-regulating genes[8] (with uncertain impact of genetic dosage on pathogenesis). There is a clear need to understand the specific lesions in immune regulatory pathways that underlie unbridled inflammation in critically ill patients with MAS and HLH to facilitate diagnosis and optimize therapy.



Pathways to hyperinflammation of antigen presenting cell (APC)/macrophage (Mac). Models of pathologic inflammation in fHLH (A) and MAS (B). (A) In fHLH, ineffective cytotoxic lymphocyte (T cell [T]) function leads to persistent activation of APC. Failure to prune activated APCs leads to production of IFN-γ, which drives the resulting cytokine storm. (B) The reports[1,2] discussed in this review identify mechanisms of MAS in which NLRC4 activation or TLR9 signaling lead to high levels of free IL-18 that stimulate macrophage (Mac) activation and production of IFN-γ, which drives the resulting cytokine storm.

In this issue of *Blood*, Weiss et al report the unique role of IL-18 in promoting MAS. In addition to systemic juvenile idiopathic arthritis (JIA) and other autoimmune diseases, inflammasomopathies, monogenic disorders caused by activating mutations in genes that regulate inflammatory cell death (ie, pyroptosis), have recently been gathered under the MAS umbrella. Specifically, activating mutations in *NLRC4* (*NLRC4*^{T337S} and *NLRC4*^{V341A}) were identified in patients with recurrent MAS, enterocolitis, and highly elevated plasma IL-18.[9,10] IL-18 has been identified among the components of the HLH cytokine storm for decades, with a first report in 1999 associating prolonged IL-18 elevation with poor outcomes.[11] However, IL-18 levels have not been among the routine laboratory tests used to observe patients with HLH/MAS, so there has not been a body of knowledge to inform the clinical relevance of IL-18 in HLH or MAS. A major finding of the Weiss et al study is that IL-18 performed well as a diagnostic biomarker, with levels >24 000 pg/mL distinguishing patients with MAS (with systemic JIA) from those with familial and presumed secondary HLH, with 83% sensitivity and 94% specificity for MAS vs familial HLH. The ratio of IL-18 to CXCL9 further enhanced the ability to discriminate between MAS, HLH, and other hyperinflammatory syndromes. Not only is IL-18 a biomarker for MAS, but mechanistic studies in mouse models demonstrated a central role in pathogenesis as well, consistent with the previous observation that a patient with NLRC4-associated MAS had significant clinical response to IL-18 blockade.[12] A remarkable finding is that the major source of pathogenic IL-18 in a mouse model with *NLRC4*^{T337S} was not lymphocytes or macrophages but rather intestinal epithelium. These mice were not cured by hematopoietic cell transplantation, potentially

a cautionary tale for some patients with refractory MAS/HLH.

The report by Girard-Guyonvarc'h et al further illuminates the mechanistic importance of IL-18 in MAS. The study extends a model of MAS published by Behrens et al[13] in which persistent TLR9 stimulation through repeated injection of cytosine guanine 1826 oligonucleotide (CpG) in a normal mouse strain led to clinical signs of MAS with elevation of IFN-γ and IFN-γ–related genes independent of lymphocyte engagement of antigen-presenting cells. Girard-Guyonvarc'h et al demonstrate that after the first CpG injection, there was an increase of IL-18 and IL-18 binding protein (IL18BP). Furthermore, IL18BP-deficient mice injected with CpG developed marked increases in IL-18, IFN-γ, CXCL9, and development of MAS that resolved with IL-18 or IFN-γ block5de.

Together, these studies demonstrate a convincing role for IL-18 in driving MAS via mechanisms independent of lymphocyte dysfunction (see figure). It is becoming clear that mechanisms differentiating fHLH and MAS extend beyond whether oncology or rheumatology is first consulted. MAS may be triggered by relentless antigen stimulation in the case of autoimmune disease or by NLRC4 activation, both resulting in exuberant IL-18 production that induces IFN-γ, at that point joining the fHLH pathway toward a cytokine storm. Although these studies support a distinction between MAS and fHLH, they also introduce a question of whether the designation MAS itself may be too broad. Future studies to dissect the genetic lesions and antigenic challenges that lead to pathological inflammation will inform opportunities to rapidly diagnose and treat the clinical fire and fury of HLH/MAS through rational manipulation of immune activation pathways.

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

REFERENCES

1. Weiss ES, Girard-Guyonvarc'h C, Holzinger D, et al. Interleukin-18 diagnostically distinguishes and pathogenically promotes human and murine macrophage activation syndrome. *Blood*. 2018;131(13):1442-1455.
2. Girard-Guyonvarc'h C, Palomo J, Martin P, et al. Unopposed IL-18 signaling leads to severe TLR9-induced macrophage activation syndrome in mice. *Blood*. 2018;131(13):1430-1441.
3. Janka GE. Familial hemophagocytic lymphohistiocytosis. *Eur J Pediatr*. 1983;140(3):221-230.
4. Grom AA, Horne A, De Benedetti F. Macrophage activation syndrome in the era of biologic therapy. *Nat Rev Rheumatol*. 2016;12(5):259-268.
5. Jordan MB, Hildeman D, Kappler J, Marrack P. An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and interferon gamma are essential for the disorder. *Blood*. 2004;104(3):735-743.
6. Bracaglia C, de Graaf K, Pires Marafon D, et al. Elevated circulating levels of interferon-γ and interferon-γ-induced chemokines characterise patients with macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. *Ann Rheum Dis*. 2017;76(1):166-172.
7. Osugi Y, Hara J, Tagawa S, et al. Cytokine production regulating Th1 and Th2 cytokines in hemophagocytic lymphohistiocytosis. *Blood*. 1997;89(11):4100-4103.
8. Kaufman KM, Linghu B, Szustakowski JD, et al. Whole-exome sequencing reveals overlap between macrophage activation syndrome in systemic juvenile idiopathic arthritis and familial hemophagocytic lymphohistiocytosis. *Arthritis Rheumatol*. 2014;66(12):3486-3495.
9. Canna SW, de Jesus AA, Gouni S, et al. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. *Nat Genet*. 2014;46(10):1140-1146.
10. Romberg N, Al Moussawi K, Nelson-Williams C, et al. Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. *Nat Genet*. 2014;46(10):1135-1139.
11. Takada H, Ohga S, Mizuno Y, et al. Oversecretion of IL-18 in haemophagocytic lymphohistiocytosis: a novel marker of disease activity. *Br J Haematol*. 1999;106(1):182-189.
12. Canna SW, Girard C, Malle L, et al. Life-threatening NLRC4-associated hyperinflammation successfully treated with IL-18 inhibition. *J Allergy Clin Immunol*. 2017;139(5):1698-1701.
13. Behrens EM, Canna SW, Slade K, et al. Repeated TLR9 stimulation results in macrophage activation syndrome-like disease in mice. *J Clin Invest*. 2011;121(6):2264-2277.

DOI 10.1182/blood-2018-02-828186

LYMPHOID NEOPLASIA

Comment on Pfeifer et al, page 1464

ARF way to Ph+ ALL stratification?

Adele K. Fielding | UCL Cancer Institute

In this issue of *Blood*, Pfeifer et al have determined that *CDKN2A/2B* deletions are "a strong and independent prognostic marker for predicting risk of relapse and overall survival" when adults with Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL) are treated with both imatinib and allogenic stem cell transplantation (aSCT).[1]

In 2018, the presence of t(9;22) in newly diagnosed ALL has positive connotations. We now know that when tyrosine kinase inhibitors (TKIs) are added immediately to the cytotoxic therapy of Ph+ ALL, the rate of complete remission (CR) reaches 95% to 100% with therapy failure being more likely because of treatment-related mortality (TRM) than therapy resistance. Indeed, convincing randomized controlled trial data[2] demonstrated that a reduction in the intensity of the initial cytotoxic therapy can significantly reduce early TRM. Now, almost 100% of patients with Ph+ ALL will reach CR and become assessable for aSCT. Can we refine our understanding of who are the best candidates? Ongoing clinical studies are addressing the question of whether patients with Ph+ ALL achieving early complete molecular remissions with later-generation TKIs can be spared the toxicity of aSCT. However, the current standard of care remains aSCT, where possible.

It has long been known that frequent, nonrandom "additional chromosome abnormalities" impact long-term outcome in Ph+ ALL; gain of a second Ph (+der 22), high hyperdiploidy, and loss of chromosomes 7, 7p, and/or 9p all impact relapse risk and survival. Loss of 9p, the locus for cyclin-dependent kinase inhibitor 2 A and B (*CDKN2A/2B*), has already been associated with a poorer relapse-free survival in 3 clinical studies of Ph+ ALL.[3-5] Pfeifer et al used single nucleotide polymorphism arrays and multiplex ligation-dependent probe amplification to uncover the impact of