Using the Extremes of Human Inflammation to Understand the Transcriptional Control of IL-18

Hemophagocytic lymphohistiocytosis (HLH) is a rare pediatric syndrome associated with inherited defects in the expression or extracellular secretion of perforin, a pore-forming protein expressed by cytotoxic CD8 T cells or natural killer cells (1). Impairments in perforin function prevent these cytolytic cells from inducing apoptosis in virally infected (or otherwise damaged) host cells, leading to a persistent nidus of innate immune activation. The consequent "cytokine storm" of HLH manifests as a febrile illness with features of multiorgan dysfunction, such as hepatitis, splenomegaly, cytopenia (especially anemia and thrombocytopenia), and central nervous system dysfunction (2). Histology of affected tissues reveals expansion of aberrantly activated T cells and macrophages that phagocytose host myeloid and erythroid cells. A diagnosis of HLH is catastrophic, with a >90% mortality at a young age in the absence of chemotherapeutics or bone marrow transplantation (3).

Although primary HLH is rare, there is increasing recognition of secondary forms of HLH (sHLH), characterized by an acquired loss of cytolytic cell function (1, 4, 5). One variant of sHLH is macrophage activation syndrome (MAS), a feared complication of pediatric rheumatologic diseases such as systemic juvenile idiopathic arthritis (sJIA) (6). Although the etiology of MAS is complex, emerging studies indicate that the chronic inflammatory activation of autoinflammatory diseases such as sJIA suppresses cytolytic cell function, potentially leading to an unremitting inflammatory response to virally infected cells (1, 6, 7). Persistent induction of macrophage activation leads to hemophagocytosis and the release of numerous proinflammatory cytokines (7, 8), mimicking the clinical findings of primary HLH.

Given that many adult patients suffer from chronic inflammatory diseases, there is increasing concern that these patients may develop MAS-like disease states when hospitalized for acute insults such as infection or malignancy (9). As such, MAS may be underrecognized in adult ICUs. There is accordingly a need to mechanistically understand the proinflammatory pathways responsible for not only the onset of sHLH/MAS but also the consequent multisystemic organ injury responsible for disease morbidity and mortality.

In this issue of the *Journal*, Verweyen and colleagues (pp. 526–539) investigate the role of IL-18 in the pathogenesis of MAS (10). IL-18 is a member of the IL-1 cytokine family, which includes IL-1β. Similar to IL-1β, IL-18 is transcribed and translated as a propeptide and then cleaved into an active form by caspases. As therapeutic inhibition of IL-1β yielded only mixed benefits in the treatment of MAS, there is increasing interest in the role of IL-18 as a potential therapeutically targetable mediator of this disease state (1). Intriguingly, Verweyen and colleagues establish the scientific premise for their investigations of MAS by studying IL-18 in a disease state at the opposite end of the inflammatory spectrum: postseptic immunoparalysis. Healthy human volunteers exposed to two sequential doses of intravenous LPS developed endotoxin tolerance, characterized by suppressed TNFα (tumor necrosis factor α), IL-6, and IL-1β release into the circulation after the second LPS exposure (11). Endotoxin tolerance was similarly induced *ex vivo* by sequential dosing of healthy human peripheral blood monocytes with LPS. In these *in vivo* human and *ex vivo* monocyte studies, the authors observed that IL-18 escaped endotoxin tolerance, contrasting the suppression of tumor necrosis factor α, IL-6, and IL-1β. The authors suggest that this escape from endotoxin tolerance may be a consequence of a uniquely delayed induction of IL-18 transcription after LPS. Although transcription of other cytokines peaked and resolved rapidly after the first LPS dose, the delayed kinetics of IL-18 transcription led to ample IL-18 mRNA availability at the time of the second LPS dose, potentially providing continued substrate for protein translation. The authors speculate that these unique IL-18 kinetics, which corroborate a recently published study of IL-18 and IL-1β by Zhu and Kanneganti (12), allow for persistent expression of an inflammatory cytokine that escapes LPS tolerance, a finding potentially relevant to unremitting auto-inflammatory states such as MAS.

After identifying delayed transcription of IL-18 after LPS, the authors sought to determine the factors responsible for these unique transcriptional kinetics. Using human peripheral blood monocytes, the authors observed that IL-18 induction was maximal after TLR4 (Toll-like receptor 4) activation, with TLR5 agonists inducing only a blunted activation of IL-18. In addition to TLR agonism, induction of IL-18 transcription required type I IFN (IFN α/β) activation of JAK/STAT signaling. Conversely, type II IFN (IFN γ) had no effect on IL-18 transcription. Type I IFN not only induced IL-18 but also controlled the kinetics of translation: pretreatment of monocytes with IFN α/β accelerated the onset of LPS-induced IL-18 transcription. These findings were confirmed using peripheral blood monocytes collected from a patient with a STAT1 gain-of-function mutation. Notably, the authors did not test whether this acceleration of IL-18 transcription reversed the previously observed ability of IL-18 to escape endotoxin tolerance. Interestingly, type I IFN/JAK/STAT signaling had an opposite, inhibitory effect on IL-1β expression in normal human peripheral blood monocytes, again demonstrating divergent mechanisms of transcriptional control of these related cytokines (12).

After using models of endotoxin tolerance to identify the unique transcriptional kinetics of IL-18, Verweyen and colleagues shifted

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their focus to investigate the effect of type I IFN/JAK/STAT/IL-18 signaling on auto-inflammatory diseases such as sJIA and MAS. In patients with sJIA or other autoinflammatory states (e.g., familial Mediterranean fever [FMF]), peripheral blood monocyte expression of IL-18 was highly correlated with expression of IFN-related genes, suggesting a mechanistic association. Furthermore, microtubule destabilizing agents such as colchicine or nocodazole, commonly used to treat autoinflammatory diseases, suppressed IL-18 and IFN β expression in LPS-treated peripheral blood monocytes. Colchicine- or nocodazole-induced suppression of IL-18 transcription could be reversed by the administration of exogenous IFN α/β. The translational relevance of these findings was supported by an observed suppression of circulating IL-18 in colchicine-treated patients with FMF.

Finally, the authors confirmed the importance of JAK/STAT signaling to IL-18 expression *in vivo* by analyzing samples collected from previously published studies of mouse models of MAS (13, 14). These models, in which a MAS-like phenotype is induced by repeated dosing with the TLR9 agonist CpG (with concurrent hemophagocytosis, if IL 10 is additionally inhibited [1]), revealed that treatment with the JAK1/2 inhibitor ruxolitinib suppressed IL-18 expression. Furthermore, treatment of a MAS human patient, who experienced a partial response to anti-IL-18 therapy (15), with the JAK1/3 inhibitor tofacitinib similarly suppressed circulating IL-18, coincident with improved clinical outcomes.

Taken together, this comprehensive work by Verweyen and colleagues elegantly used pathologic extremes of human inflammation, ranging from postseptic immunoparalysis to fulminant autoinflammatory disorders such as MAS and FMF, to glean new insights into the transcriptional control of IL-18. Similar to most important studies, there remain numerous unanswered questions. In contrast to IFN α/β signaling, the authors found that IFN γ, a cytokine with known importance to MAS pathogenesis (1), exerted minimal impact on IL-18 signaling. These findings demonstrate that the complex pathophysiology of these autoinflammatory conditions likely cannot be explained by IL-18 alone. Furthermore, it is uncertain if (and how) the authors' work, derived largely from *ex vivo* studies of LPS-treated peripheral blood monocytes, can be extrapolated to inform the *in vivo* behavior of CD8 T cells and/or hemophagocytic tissue-resident macrophages pathognomonic of MAS. Nevertheless, this work provides exciting insights into the mechanisms responsible for control of IL-18 expression while identifying therapeutic targets (e.g., type I IFN, JAK/STAT signaling) that may potentially help patients with autoinflammatory disease. ■

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