Dousing fire with gasoline: interplay between lysosome damage and the NLRP3 inflammasome. Focus on “NLRP3 inflammasome signaling is activated by low-level lysosome disruption but inhibited by extensive lysosome disruption: roles for K+ efflux and Ca2+ influx”

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Since its discovery over a decade ago, the NLRP3 inflammasome has been the focus of intense investigation. Although much has been learned about the molecular regulation of this potent inflammatory complex, there are several unanswered questions about what drives its assembly. At its simplest, the inflammasome is a cytosolic complex of proteins that functions to cleave and release what drives its assembly. At its simplest, the inflammasome is a cytosolic complex, there are several unanswered questions about what drives its assembly. At its simplest, the inflammasome is a cytosolic complex of proteins that functions to cleave and release the cytokines IL-1β and IL-18 during inflammation. Inflammasome activation is tightly controlled and requires two distinct signals for complex activation and IL-1β release. Signal 1 is a priming step typically delivered by Toll-like receptor activation, and signal 2 is a cytosolic stress signal that triggers assembly of a multiprotein complex that includes the molecules NLRP3, ASC, and caspase 1 (6). In the case of the NLRP3 inflammasome, signal 2 can come in many flavors including ATP-induced ion fluxes, mitochondrial oxidative stress, and lysosome damage (3). The diverse nature of signal 2 stimuli that promote NLRP3 inflammasome assembly suggests that common upstream pathways must be engaged. The precise mechanisms through which this occurs remain poorly understood.

Although mutations in components of the inflammasome are associated with rare inflammatory diseases such as cryopyrin-associated periodic fever syndromes (CAPS), there is growing awareness of common human diseases that are also associated with inflammasome activation (3). Among the clinical disorders associated with NLRP3 inflammasome activation are gout, silicosis, atherosclerosis, Alzheimer’s disease, and diabetes (1). Interestingly, the mechanism of inflammasome activation in the majority of these conditions appears to occur as a consequence of lysosome damage. However, significant controversy exists regarding how loss of lysosome integrity causes NLRP3 assembly. Cathepsin release has been implicated in this process, but much of this data comes from the use of chemical inhibitors. Genetic models of cathepsin loss-of-function have yielded more complicated results (7). Moreover, the idea that lysosome damage is an essential process upstream of NLRP3 assembly has also been challenged (5).

Leu-Leu-O-methyl ester (LLME) is a lysosome-damaging compound that has proven to be a useful experimental tool for investigating the relationship between lysosome damage, cell death, and inflammasome activation. Upon entering a cell, LLME is trafficked to the lysosome where it is cleaved by cathepsin C to lysomotropic polymers that destabilize the lysosome membrane (9). The rapid induction of lysosomal membrane permeability (LMP) makes LLME an ideal stimulus to produce homogeneous lysosome damage and thereby facilitate time course studies to dissect the sequence of events involved in inflammasome activation.

In this issue of American Journal of Physiology-Cell Physiolo- gy, Katsnelson et al. (4) use dose escalation of LLME in bone marrow-derived dendritic cells (BMDCs) to provide new insights into regulation of NLRP3 activation by lysosome damage. The primary finding presented in this study is that the degree of lysosome permeabilization is a critical factor that controls NLRP3 inflammasome activation (Fig. 1). Low-dose LLME causes mild LMP and strongly activates the inflammasome. Paradoxically, higher doses of LLME, which produces massive LMP, actually suppresses inflammasome activation. In contrast, the cell death response to LLME is present at both high and low concentrations. Another important finding from this study was that high-dose LLME could also dampen the NLRP3 inflammasome response to other classical activators such as nigericin, suggesting a dominant-negative affect on NLRP3 complex assembly. Although others have reported that massive LMP can lead to degradation of inflammasome components including caspase 1, the authors did not observe any differences in caspase 1, ASC, NLRP3, or pro-IL-1β protein levels with high-dose LLME in BMDCs (5).

This begs the question, how does massive LMP inhibit NLRP3 inflammasome assembly?

Potassium efflux is required for NLRP3 activation and it occurs as an early event after LMP. However, potassium flux was not differentially affected in response to high- or low-level LMP, arguing that modulating this pathway does not account for inflammasome inhibition. Calcium influx also occurs in response to LMP and can modulate inflammasome activation. Interestingly, calcium influx was greatest at low doses of LLME and suppressed at higher concentrations, suggesting that reduced cytosolic calcium may explain this phenotype. However, removal of calcium from the culture media actually enhanced IL-1β release from BMDCs, arguing against a central role for calcium flux. Instead the authors demonstrate that differential ubiquitination of NLRP3 may be a factor in this response. NLRP3 is ubiquinated at baseline and this posttranslational modification prevents efficient inflammasome assembly (8). Consistent with this, treatment with a deubiquitination enzyme inhibitor strongly suppressed IL-1β release in response to LMP. In BMDCs, high-dose LLME promoted NLRP3 ubiquitination when compared with low-dose LLME, and the effects of the deubiquitinase (DUB) inhibitor were less pronounced. Together these findings suggest that high-level LMP leads to increased NLRP3 ubiquitination, which suppresses complex formation. Although it is not clear how LMP alters NLRP3

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ubiquitination, these observations explain the contradictory results reported using LLME as a stimulus for LMP and NLRP3 activation and provide mechanistic insight into the mode of inflammasome suppression by massive lysosome damage.

Although the current study by Katsnelson et al. adds to our mechanistic understanding of the complex relationship between lysosome damage and NLRP3 inflammasome activation, there are some limitations that merit discussion. Most importantly, the data presented come from a single in vitro culture system using a nonphysiologic stimulus to induce lysosome damage. In fact, stimulation of BMDCs with alum or uric acid crystals to produce LMP did not completely recapitulate the bell-shaped dose curve for IL-1β release that was seen with LLME. Therefore, it is unknown whether the gradations of lysosome damage that can be induced with LLME actually occur during human disease. Another consideration is the forgotten sibling of IL-1β, IL-1α. In contrast to IL-1β, which requires caspase 1 cleavage, IL-1α is released upon cell death or calpain cleavage (2). In the context of massive LMP, cell death still occurred, and even though the authors did not report data on IL-1α, it is likely that this cytokine was still released. This is relevant because both IL-1α and IL-1β activate the same receptor and therefore IL-1α release could potentially compensate for the loss of IL-1β in some instances of lysosome damage. Additional cell culture and animal model approaches will be necessary to evaluate these issues.

It is now clear that the NLRP3 inflammasome is involved in the pathogenesis of several common and important diseases. Therefore, understanding the molecular mechanisms that regulate its assembly is highly relevant to the advancement of human health. The lysosome appears to serve as a signaling nexus for inflammation and the nuances of this interplay are highlighted by Katsnelson et al. Further investigation will be necessary to determine whether the lysosome could serve as a therapeutic target for diseases associated with excessive inflammasome activation.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author.

**AUTHOR CONTRIBUTIONS**

J.D.S. drafted manuscript.

**REFERENCES**