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Turning on and off NLRP3

The exact molecular mechanisms that govern the activation and regulation of inflammasomes (multiprotein complexes that mediate the processing of interleukin-1β (IL-1β) and IL-18) are not fully understood. Two studies now describe new mechanisms involving Ca^{2+}, cyclic AMP and nitric oxide (NO) that activate and control the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome.

Previous studies have implicated a role for extracellular cations in the regulation of the NLRP3 inflammasome, so Lee *et al.* focused on the role of Ca^{2+} in inflammasome activation. They found that exposure of lipopolysaccharide (LPS)-primed bone marrow-derived macrophages (BMDMs) to extracellular Ca^{2+} induced IL-1β secretion through the calcium-sensing receptor (CASR). This effect of Ca^{2+} was specific for the NLRP3 inflammasome and did not activate the AIM2 or NLRC4 inflammasomes. Of note, CASR is also required for IL-1β processing in response to other NLRP3 activators.

Further experiments elucidated the molecular mechanism by which CASR activates the NLRP3 inflammasome. Indeed, CASR was shown to activate phospholipase C, which generates inositol-1,4,5-trisphosphate ($InsP_3$). By binding to its receptor (namely, $InsP_3R$), $InsP_3$ promotes the release of Ca^{2+} from endoplasmic reticulum stores into the cytoplasm. The presence of Ca^{2+} in cell-free lysates increased the association of NLRP3 with the inflammasome component ASC.

CASR also inhibits adenylyl cyclase and thus reduces cAMP levels. An inverse correlation was observed between the levels of cAMP and active IL-1β in LPS-primed BMDMs. Increased cAMP levels inhibited IL-1β secretion through the interaction of cAMP with the nucleotide-binding domain of NLRP3, which prevented inflammasome assembly. Interestingly, the mutations in NLRP3 that cause cryopyrin-associated periodic syndromes (CAPS; which are associated with spontaneous inflammasome activation) are most commonly located in this domain. The binding of cAMP to mutant NLRP3 was substantially decreased compared with its binding to wild-type NLRP3. Moreover, the uncontrolled IL-1β production by cells from patients with CAPS was attenuated by pharmacologically increasing cAMP levels in these cells, as well as by blocking $InsP_3$-mediated intracellular Ca^{2+} signalling pathways. So, Ca^{2+} and cAMP are important regulators of NLRP3 inflammasome assembly that have crucial roles in the pathogenesis of CAPS.

Although IL-1β is an important innate effector molecule, IL-1β-initiated inflammatory responses can cause widespread tissue damage. So, IL-1β production must be tightly regulated, especially in the context of persistent infections, for example with *Mycobacterium tuberculosis*. In the other study, Mishra *et al.* found that mice develop a distinct, severe pathology in response to *M. tuberculosis* infection in the absence of B and T cells, inducible nitric oxide synthase (iNOS) or interferon-γ (IFNγ). This pathology was characterized by neutrophil infiltration in tuberculosis lesions and high levels of IL-1β.

To eliminate the effect of differences in bacterial numbers between wild-type and knockout mice, the authors developed an infection model in which *M. tuberculosis* replicates only in the presence of streptomycin. Using this model, IFNγ-induced NO was shown to have a specific role in regulating the production of active IL-1β, independently of the role of NO in restricting bacterial growth.

Further analyses showed that IFNγ inhibited NLRP3-dependent processing of IL-1β in *M. tuberculosis*-infected macrophages. However, IFNγ did not suppress AIM2- or NLRC4-dependent IL-1β production. This effect of IFNγ was mediated by NO, which altered NLRP3 inflammasome assembly via *S*-nitrosylation of NLRP3, and this was sufficient to suppress inflammasome assembly and the processing of IL-1β. So, inflammasome inhibition by the adaptive immune system via IFNγ-induced NO represents an important mechanism to prevent excessive tissue damage associated with persistent infection.

Together, these studies further our understanding of NLRP3 inflammasome assembly and identify potential therapeutic avenues for the treatment of CAPS and persistent infections.

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> “IFNγ-induced NO was shown to have a specific role in regulating the production of active IL-1β”

ORIGINAL RESEARCH PAPERS Lee, G.-S. *et al.* The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca^{2+} and cAMP. *Nature* 11 Nov 2012 (doi:10.1038/nature11588) | Mishra, B. B. *et al.* Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1β. *Nature Immunol.* 18 Nov 2012 (doi:10.1038/ni.2474)