hypomethylation phenotype. The potential role for Stat5 in guiding Tet binding to the *Foxp3* locus is of particular interest given recent findings demonstrating that IL-2 sensing and Stat5 interactions with the CNS2 region are important for the maintenance of Treg cell identity (Feng et al., 2014).

Acquisition of the proper epigenetic modifications is critical for the establishment of a stable Treg cell lineage, and elucidating the signals and molecular mechanisms that regulate the Treg cell epigenome are of fundamental importance. The findings reported by Yang et al. (2015) provide novel insight into how signaling by the gasotransmitter H_2S influences DNA demethylation of key *Foxp3* regulatory regions. The production of H_2S requires substrates generated by the metabolism of methionine, indicating a role for nutrient sensing in regulating the Treg cell epigenome. In the context of previous work in the field, these findings indicate that the integration of environmental signals (antigen, cytokine, and nutrients) allows for the dynamic regulation of Treg cell identity. Indeed, the tunable nature of these signals might allow for more precise regulation of Treg cell identity and function.

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POPsicle for Fever! Cooling Down the Inflammasome

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Inhibition of the inflammasome might be beneficial for numerous inflammatory pathologies. In this issue of *Immunity*, de Almeida et al. (2015) report that the PYRIN domain-only protein (POP1) efficiently inhibits inflammasome activation, identifying it as a pan-inflammasome inhibitor.

In recent years the inflammasome and interleukin 1 (IL-1) signaling have been centrally implicated in many human pathologies. This includes a wide range of chronic inflammatory disorders such as diabetes, atherosclerosis, rheumatoid arthritis, and obesity, among others (Guo et al., 2015). In addition to these multifactorial chronic disorders, cryopyrinopathies (Cryopyrin-associated periodic syndromes [CAPS]) are the result of mutations that cause constitutive inflammasome activation and unchecked IL-1 signaling (Broderick et al., 2015). This has led to an explosion of clinical trials based on inhibiting IL-1-mediated pathologies, including the use of an IL-1 receptor antagonist (Anakinra/Kineret), an IL-1 receptor decoy (Rilanocept), and various monoclonal antibodies against IL-1β (Dinarello et al., 2012; Guo et al., 2015). In addition to these therapeutic modalities, new small molecule inhibitors targeting various steps in the inflammasome pathway have been developed with promising results (Guo et al., 2015). Many of these therapeutics target signaling downstream of IL-1, and even those that target upstream events such as inflammasome activation generally target only a specific type of inflammasome rather than all inflammasomes (Guo et al., 2015).

The inflammasome is a multi-component structure, which, when formed, activates caspase-1, which in turn can cleave the inactive precursors of specific pro-inflammatory cytokines (mainly IL-1β and IL-18), thereby activating them and leading to their secretion (Guo et al., 2015). The best studied of the inflammasomes, and the one targeted by most current therapeutics, is the nucleotide binding domain and leucine-rich repeat (NLR) pyrin domain containing 3 (NLRP3) inflammasome, which, not coincidently, is associated with the largest number of inflammasome-mediated diseases (Guo et al., 2015; Man and Kanneganti, 2015). The NLRP3 inflammasome responds to many diverse danger signals including, but not limited to, extracellular ATP, crystals and other insoluble particles, and bacterial toxins (Guo et al., 2015; Man and Kanneganti, 2015). Although not

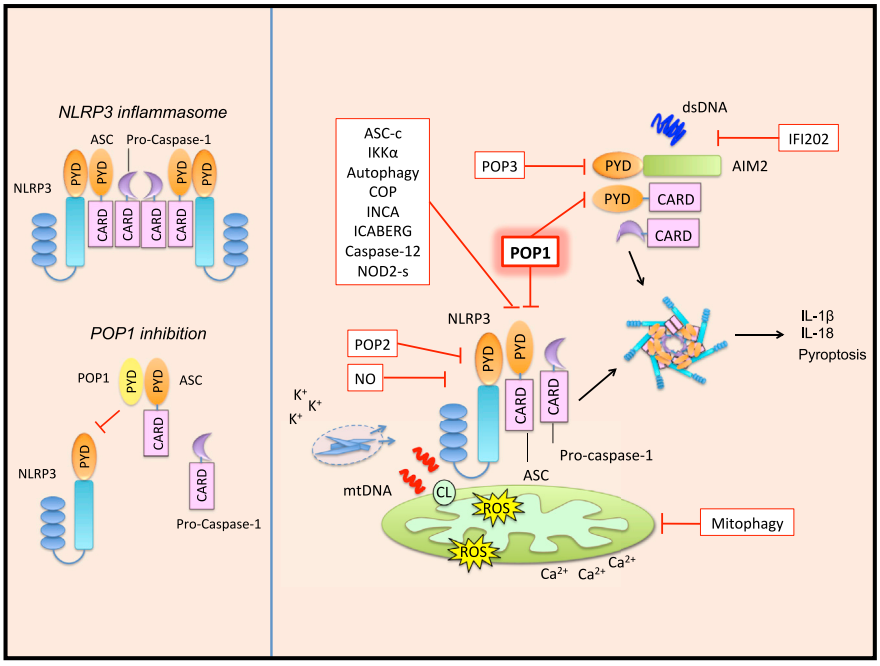

Figure 1. Mechanisms of Inflammasome Inhibition
Left: POP1 binds to the PYD of ASC and prevents PYD-PYD interactions between ASC and NLRP3. Right: multiple inflammasome inhibitory mechanisms are shown, including POP1.

fully elucidated, these dangers signals all seem to converge at a central hub involving mitochondria and/or K^+ efflux (Guo et al., 2015; Man and Kanneganti, 2015). Additionally, lysosomal disruption can lead to NLRP3 activation, but whether or not this is independent of mitochondrial/K^+ involvement is not well understood (Guo et al., 2015; Man and Kanneganti, 2015). Other common NLR inflammasomes include NLRP1 and NLRC4, which respond to certain bacterial toxins and flagellin, respectively (Guo et al., 2015; Man and Kanneganti, 2015). Additionally, the AIM2 inflammasome activates caspase-1 in response to bacterial DNA (Guo et al., 2015; Man and Kanneganti, 2015).

Inflammasome assembly requires multiple components being recruited in an ordered structure with prion-like growth properties (Guo et al., 2015; Man and Kanneganti, 2015). In the case of NLRP3, the key adaptor molecule ASC bridges the gap between NLRP3 and Caspase-1 by binding to their PYRIN and CARD domains, respectively (Guo et al., 2015; Man and Kanneganti, 2015). ASC is also required for both the AIM2 and NLRP1 inflammasome activation in a similar capacity (Guo et al., 2015; Man and Kanneganti, 2015). Interestingly, although NLRC4 has its own CARD domain and therefore theoretically doesn't require ASC to bind Caspase-1, new data suggest that ASC might still be required for full activation (Guo et al., 2015). Thus, ASC plays a central role in inflammasome activation, and its inhibition could affect multiple inflammasome pathways.

In the article by de Almeida et al. (2015) appearing in this issue of *Immunity*, the authors have identified PYRIN (PYD) only protein (POP1) as a negative regulator of inflammasome activation. Although the POP family of proteins was identified in humans more than 10 years ago (they are not found in mice) and includes POP1 (also known as ASC2), POP2, POP3, and POP4 (Stehlik et al., 2003), their functions have been unclear. Recently, POP3 was found to interact with AIM2 and inhibit AIM2 inflammasome activation (Khare et al., 2014). POP1 was initially suspected to enhance NLRP3 inflammasome activation (Stehlik et al., 2003), but more recently Atianand and Harton (2011) reported that POP1 does not alter either ASC or NLRP3 processing of IL-1β. Thus, the function of POP1 remained controversial. However, the current work by de Almeida et al. (2015) now clearly demonstrates that POP1 knockdown promotes LPS-induced IL-1β secretion in both THP1 cells and primary human macrophages. The authors showed by immunoprecipitation that POP1, which contains a PYRIN domain but lacks a CARD domain, prevented NLRP3 from interacting with ASC, thus inhibiting inflammasome oligomerization (Figure 1, left). Consistent with this, POP1 expression diminished IL-1β secretion in response to LPS. Importantly, the inhibitory effects of POP1 were observed not only for NLRP3, but also for AIM2 and NLRC4 inflammasome activation. Despite the fact that NLRC4 has its own CARD domain, which can bind to caspase-1 directly, POP1 inhibited flagellin-driven inflammasome activation. This suggests that ASC is required for full NLRC4-driven inflammasome activation by stabilizing the structure, as has been reported previously (Guo et al., 2015). Furthermore, the authors found that POP1 overexpression inhibited ASC particle release, thereby preventing secondary inflammasome activation after engulfment by macrophages. de Almeida et al. (2015) generated transgenic mice expressing human *POP1* under the macrophage-specific CD68 promoter and showed that NLRP3 inflammasome autoactivation by CAPS mutation was significantly inhibited. More importantly, these mice were protected against LPS-induced peritonitis.

The authors also found that POP1 was regulated in an NF-κB-dependent manner, suggesting that its expression in human macrophages is part of a feedback inhibitory loop. Considering the number and variety of chronic inflammatory disorders mediated by IL-1 signaling, it is not surprising that the inflammasome and IL-1 signaling are tightly regulated (Dorfleutner et al., 2015; Guo et al., 2015; Man and Kanneganti, 2015). Endogenously, we have evolved many checkpoints and feedback mechanisms to prevent overactive immune signaling, especially through the inflammasome and/or IL-1 signaling pathway (Figure 1, right). This includes two-step activation of the inflammasome, autophagy in opposition to NLRP3 activation, ASC phosphorylation, IL-1 receptor antagonist and IL-1 receptor 2, alternatively spliced products such as MyD88s, and A20 ubiquitination of TRAF6, among others (Garlanda

et al., 2013; Guo et al., 2015). The authors' investigation into the inhibitory activity of POP1 is especially important because their data suggest that POP1 inhibits all the major inflammasomes (NLRP3, NLRC4, and AIM2), thus having a broad anti-inflammatory effect.

The authors found that POP1 was regulated by NF-κB in human macrophages, but it is intriguing that they also observed that POP1 was downregulated in CAPS patients, which seems counter-intuitive but might explain why CAPS patients are highly susceptible to increased systemic autoinflammation. Although the authors suggest that these patients might also have a deleterious mutation leading to POP1 downregulation, the likelihood for these patients to have a loss-of-function SNP for POP1 in addition to the gain-of-function SNP in NLRP3 seems low and will require further study. More likely, other as-yet-to-be-discovered feedback mechanisms are at play that leads to reduction in POP1 expression in CAPS and sepsis patients. Importantly, this novel finding led the authors to generate a fusion protein of POP1 and the HIV peptide TAT that could be delivered into any cell with the promise that it would target and inhibit the activation of all the inflammasomes. Indeed, mice given the TAT-POP1 fusion protein intraperitoneally were protected against LPS-induced peritonitis.

The work by de Almeida et al. (2015) provides compelling evidence for a new feedback regulatory loop to control excessive inflammasome activation. However, more work remains to be done; the endogenous regulation of POP1 is still unclear, and questions remain as to why CAPS patients downregulate POP1 expression. In addition to POP1, human cells also possess four ASC alternatively-spliced isoforms (ASC, ASC-b, ASC-c, and ASC-d), whereas mouse macrophages have only ASC and ASC-c (Dorfleutner et al., 2015). In contrast to ASC and ASC-b, ASC-c is structurally missing part of the PYD and functions as an inhibitor of inflammasome activation. Further studies are required to better understand how POPs regulate inflammasome activation and if and how ASC alternative splicing might further fine-tune the control of inflammasome activation.

The use of TAT-POP1 as a potential novel therapeutic has tremendous translational value and is very appealing. Fusing the HIV TAT peptide to the protein of choice allows for efficient entry into mammalian cells. Because POP1 theoretically targets all of the inflammasomes that require ASC, this makes POP1 an attractive candidate as a powerful pan-inflammasome inhibitor for numerous inflammatory diseases. Although most attempts at disrupting the inflammasome-induced inflammatory pathologies have targeted downstream IL-1 signaling, newer small molecule inhibitors working upstream are currently being evaluated (Guo et al., 2015; Man and Kanneganti, 2015). Attempts to develop therapeutics based on caspase-1-specific peptidomimetic inhibitors (pralnacasan and VX-765) based on the YVAD tetrapeptide sequence are providing mixed results because of liver toxicity. Other new inhibitors target only a specific inflammasome (i.e., NLRP3) (Guo et al., 2015; Man and Kanneganti, 2015). Therefore, TAT-POP1, which seems to be a pan-inflammasome inhibitor, might provide an advantage and have greater clinical utility. Additionally, unlike therapeutics that target IL-1 signaling, interfering with inflammasome activation is a more proximal event and is likely to lead to greater protection. Indeed, inflammasome activation is often associated with cell death (pyroptosis and/or apoptosis) and POP1 efficiently prevented cell death in addition to inhibiting inflammasome activation. Thus, the best way to cool down inflammation might be with a POP1(sicle).

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