JLB

Editorial

Editorial: **Flexible Syk: turning on and off the inflammasome as needed**

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RECEIVED DECEMBER 27, 2014; REVISED FEBRUARY 10, 2015; ACCEPTED FEBRUARY 25, 2015. DOI: 10.1189/jlb.2CE1214-627R

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The detection of microbial products is key to activation of the innate and adaptive immune system and ultimately, to survival of the host. In the innate immune system, microbial products are recognized by PRRs. These receptors make up a family of signaling receptors that recognize PAMPs, which are conserved structures found almost exclusively on microbes. Within the family of PRRs is the NLR family, which is composed of cytosolic receptors that recognize intracellular microbes and danger signals. Several members of the NLR family activate inflammatory caspases via the formation of inflammasomes: a multiprotein complex composed of an NLR family member, the adaptor protein ASC, and the cysteine protease procaspase 1 [1]. Formation of the inflammasome results in cleavage and activation of caspase 1, which is important for host defense by proteolytically activating IL-1β and IL-18, and induction of an inflammatory cell death process called pyroptosis. Activated caspase 1 has other substrates, such as cytoskeletal proteins and enzymes, of the glycolytic pathway that suggest that it may play important roles in a wider spectrum of cell functions [2].

Abbreviations: ASC = apoptosis-associated speck-like protein containing a caspase activation and recruitment domain, BMDC = bone marrow-derived dendritic cell, BMDM = bone marrow-derived macrophage, CARD = caspase activation and recruitment domain, DAMP = damage associated molecular pattern, Hz = hemozoin, MSU = monosodium urate, NLR = nucleotide-binding oligomerization domain-like receptor, PAMP = pathogen-associated molecular pattern, PRR = pattern recognition receptor, SYK = spleen tyrosine kinase

Members of the NLR family that are involved in the formation of inflammasomes include NLRC4, NLRP1, and NLRP3. Of these, only NLRP3 is activated by a diverse group of stimuli that includes PAMPs and DAMPs, such as extracellular ATP, MSU, and silica. Because these stimuli are structurally and chemically diverse, it is unlikely that they all activate NLRP3 by directly binding to it; rather, they may converge on a common molecule/pathway that leads to NLRP3 activation and subsequently, formation of the inflammasome. There are several common terminal pathways that have been hypothesized to activate NLRP3, including lysosomal disruption, mitochondrial reactive oxygen species generation, and K^+ efflux. Activation of NLRP3 results in its interaction with ASC via the pyrin domain, resulting in self-oligomerization of ASC into macromolecular structures, termed ASC specks, which recruit procaspase 1 via interactions with their respective CARDs, resulting in autocleavage and activation of caspase 1. Activated caspase 1 cleaves pro-IL-1β or pro-IL-18, resulting in the secretion of mature IL-1β and IL-18 [3].

The importance of tight regulation of NLRP3 inflammasome activation has been demonstrated by its pathophysiologic role in many inflammatory disorders. Naturally occurring mutations in the *nlrp3* gene result in a collection of autoinflammatory syndromes termed cryopyrin-associated periodic syndrome. The gain-of-function mutations result in a constitutively active NLRP3 inflammasome and increased IL-1β production, which is linked to pathogenesis [4]. Aberrant NLRP3 inflammasome activation has also been linked to other inflammatory disorders, including type 2 diabetes, atherosclerosis, gout, and Alzheimer's disease. Therefore, it is critical that activation of the NLRP3 inflammasome is tightly regulated to prevent damage to the host. There are 2 steps required for activation of the NLRP3 inflammasome in macrophages: a priming step to increase expression of NLRP3 (and pro-IL-1β) and a 2nd step to activate NLRP3 and induce formation of the inflammasome. Each step is regulated by multiple mechanisms; for instance, during the priming step, PRR stimulation of the NF-κB pathway results in an increase in NLRP3 gene transcription. PRR stimulation also results in post-translational regulation of NLRP3 protein via deubiquitylation of NLRP3, generating an increase in intracellular levels of the protein (reviewed in ref. [5]). However, it is clear that there are additional signaling molecules and pathways that regulate NLRP3 inflammasome activation. Recent studies have suggested that kinases play a role; however, there is a lack of information on the mechanisms by which specific kinases directly regulate inflammasome activation. The identification of these regulatory pathways is critical for the development of therapeutics to treat the numerous inflammatory disorders that are associated with excess IL-1β. SYK is a nonreceptor tyrosine kinase that interacts with ITAM-containing proteins and receptors. SYK is activated following stimulation of several PRRs, in particular,

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the C-type lectin receptors, and is critical for the induction of proinflammatory cytokine gene expression. However, there are conflicting reports on the extent to which SYK is involved in activation of the NLRP3 inflammasome [6–8].

In this issue of *Journal of Leukocyte Biology*, Lin et al. [9] begin to tease apart the mechanisms by which SYK regulates NLRP3 inflammasome activity in primary macrophages following stimulation with various NLRP3 stimuli. The authors demonstrate that SYK is phosphorylated following stimulation by each of the NLRP3 activators: ATP, nigericin (microbial toxin), MSU, and alum. Inhibition of SYK activity by use of chemical inhibitors (after the cells had already been primed with LPS) inhibited caspase 1 activation and the production of IL-1β. These results were confirmed by use of SYK-deficient macrophages that also exhibited less caspase 1 activity and IL-1β production following stimulation with each of the activators. These results clearly demonstrate a positive regulatory role for SYK in NLRP3 inflammasome activation in macrophages.

To begin to dissect the mechanism by which SYK regulates NLRP3 inflammasome activation, the authors focused on identifying specific interactions between SYK and inflammasome components. The first step was to show that SYK was required for ASC oligomerization, which is necessary for activation of caspase 1. The absence of SYK (by use of genetic and chemical approaches) resulted in inhibition of ASC oligomerization following stimulation with each of the NLRP3 activators (ATP, alum, nigericin, or MSU). This suggested that SYK is recruited to the NLRP3 inflammasome complex following stimulation, and the authors used a series of coimmunoprecipitation studies to test this hypothesis. With the use of antibodies to SYK or ASC for the immunoprecipitation, the authors demonstrated that SYK associated with NLRP3 and ASC following ATP or alum stimulation. Importantly, in the absence of SYK, there was a reduced association between ASC and procaspase 1, which is required for triggering caspase1 activation. The domain in SYK responsible for interaction with NLRP3 and ASC was determined to be the C-terminal kinase domain. Altogether, these results demonstrate that SYK interacts directly with components of the NLRP3 inflammasome, specifically NLRP3 and ASC, and this interaction promotes the association of ASC and procaspase 1. Lin and colleagues [9] then used an in vitro SYK kinase assay to demonstrate that SYK phosphorylates ASC and NLRP3. The authors mutated predicated SYK phosphorylation sites in ASC and demonstrated that ASC mutants with tyrosine residues 146 and 187 mutated to phenylalanine were no longer phosphorylated by SYK. More importantly, mutations of these residues inhibited ASC oligomerization and subsequently, caspase 1 activation and IL-1β production. The results clearly suggest that SYK phosphorylation of ASC on these residues is important for ASC oligomerization and subsequently, caspase-1 activation. The authors' data corroborate and extends a previous report, which found a positive regulatory role for SYK in NLRP3 inflammasome activation and IL-18 production following stimulation with ATP or alum in peritoneal macrophages [7]. Stimulation of PMA-differentiated THP cells and BMDMs with Hz (crystalline product formed during *Plasmodium* infection) activates the inflammasome, resulting in IL-1β production, and chemical inhibition of SYK resulted in a decrease in mature IL-1β [6]. Taken together, these studies suggest that in macrophages, stimuli that directly activate the inflammasome (ATP, alum, Hz, nigericin, and MSU) phosphorylate and activate SYK, allowing it to interact with and phosphorylate ASC, resulting in ASC oligomerization, inflammasome formation, and caspase 1 activation (**Fig. 1A**). There is considerable heterogeneity among macrophage populations, and although these studies were performed with macrophages from different sources, it is still unclear whether SYK regulation of the NLRP3 inflammasome will be similar in macrophages isolated from other tissues and species. Another important question is whether SYK regulation of the inflammasome is cell-type specific. Gross et al. [8] found no role for SYK in NLRP3 inflammasome activation, following stimulation of BMDCs with nigericin. This difference may reflect differential regulation of the NLRP3 inflammasome and/or differential expression and regulation of SYK activity between macrophages and dendritic cells.

Further complicating the issue of SYK regulation of the inflammasome is the complexity of the stimulators. Microbes have multiple PAMPs that stimulate numerous PRRs, some of which also require SYK for their activity. Activation of SYK by upstream receptors may dictate the extent to which SYK is available to regulate inflammasome activation directly. Alternatively, activation of SYK by upstream receptors may regulate the inflammasome by inducing or inhibiting expression of pro-IL-1β or NLRP3. To begin to investigate this additional complexity, the authors wanted to determine the net effect of SYK on IL-1β production when SYK is activated by TLR4 signaling during the priming stage. In previous studies, Lin et al. [10] had demonstrated that SYK negatively regulates LPS-induced signaling pathways. In the current paper, LPS stimulation alone resulted in an increase in pro-IL-1β and NLRP3 gene expression in SYK-deficient macrophages compared with wild-type. With the addition of NLRP3 stimuli (ATP, alum, MSU, or nigericin), there was also an increase in the amount of IL-1β expression in the SYK-deficient macrophages. These intriguing results suggest that the outcome of SYK regulation of the inflammasome may be dictated by upstream receptors that use SYK for activation of their signaling pathways (Fig. 1B). However, these results are in contrast with studies by He et al. [11], who found that SYK-deficient BMDMs had reduced IL-1β production following LPS + ATP (or nigericin) and an increase in IL-18. These studies differ in their source of macrophages; the SYK-deficient BMDMs were derived from chimeric mice, which might contribute to the differing results. These results are also in contrast with studies investigating the role of SYK in inflammasome activation by fungi and bacteria containing PAMPs that interact with C- type lectin receptors. SYK was necessary for induction of pro-IL-1β and subsequently, secretion of mature IL-1β in a human monocyte cell line following stimulation by *A. fumigatus* [12]. *C. albicans* also required SYK to stimulate pro-IL-1β production in BMDCs [8]. In this study, SYK was also necessary for caspase 1 activation, suggesting that SYK

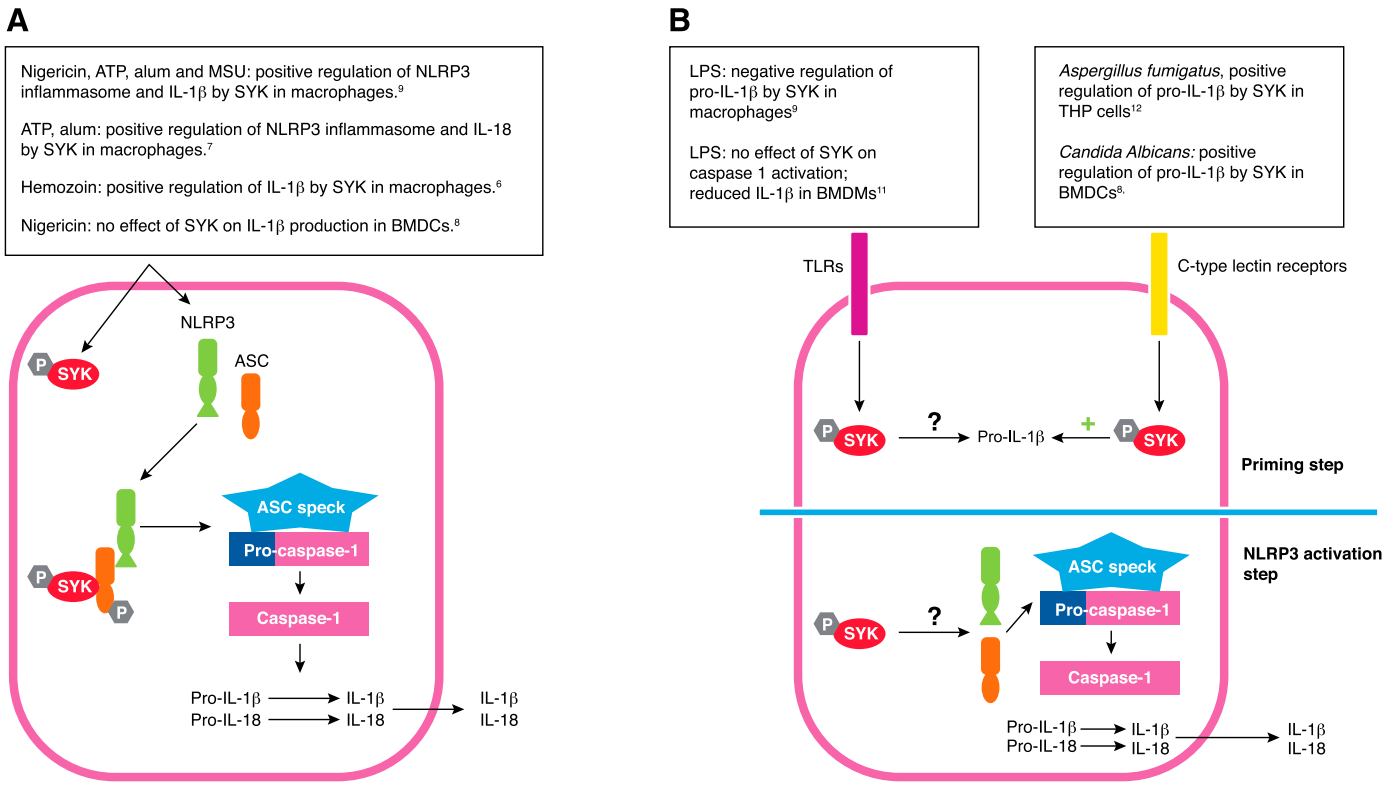

Figure 1. Summary model for proposed SYK regulation of NLRP3 inflammasome. (A) Activators of the NLRP3 inflammasome, such as ATP and alum, phosphorylate SYK, which then binds and phosphorylates ASC at tyrosine residues 146 and 187 [9]. Phosphorylation of ASC triggers oligomerization, formation of the inflammasome complex, and activation of caspase 1, resulting in cleavage of pro-IL-1β or pro-IL-18. (B) SYK plays a differential role in pro-IL-1β production during the priming step. Stimulation through TLR4 by LPS revealed a negative regulatory role for SYK in pro-IL-1β production [10]. Alternatively, stimulation of cells with microbes that can interact with C-type lectin receptors has demonstrated a positive regulatory role for SYK in pro-IL-1β production [8, 11]. Additional studies are necessary to determine whether SYK also phosphorylates and interacts with ASC during the NLRP3 activation step in these situations or if the effect of SYK on mature IL-1β production is restricted to the priming step. P, PO_4 (in gray background), Superscript numbers in text boxes refer to references.

regulation of the inflammasome by *Candida* is not restricted to regulation of pro-IL-1β production.

There are several key questions that remain to be answered. Additional studies are necessary to identify whether SYK regulation of IL-1β production by microbes is restricted to the priming step and/or the inflammasome-activation step. Recruitment of SYK to membrane receptors may effectively sequester SYK and prevent it from interacting with ASC and subsequently, regulating inflammasome activation. What is the role of SYK in regulating other products of caspase 1? The majority of studies investigates the role of SYK in IL-1β production, which requires a priming step; however, pro-IL-18 is expressed at constitutive levels and does not require a priming step. The studies by He et al. [11] suggest that SYK may differentially regulate the 2 cytokines. Is SYK involved in regulating IL-18 production (or pyroptosis) following stimulation of TLRs or C-type lectin receptors by microbes? Another key question is what dictates the positive or negative regulation of the inflammasome by SYK when multiple PRRs are activated by a microbe? Many microbes simultaneously activate TLRs and C-type lectin receptors; how does cross-talk between signaling pathways affect the outcome of SYK regulation of the inflammasome? Finally, what role do other tyrosine kinases, as well as phosphatases, play in regulating NLRP3 inflammasome activation?

SYK adds an additional level of regulation on a signaling pathway that is critical for host defense yet has the potential for causing considerable damage to the host when unchecked. The complete mechanisms by which SYK (and other tyrosine kinases) regulate the NLRP3 inflammasome are still unanswered, and the results from this paper begin to clarify the role of SYK in macrophages. Further research into this area is necessary, as it will perhaps open the door to development of novel therapeutics to treat a spectrum of inflammatory disorders, as well as therapeutics to enhance host defense to pathogens.

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KEY WORDS:
IL-1β tyrosine kinase