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COMMENTARY

Kinases: a remote control in inflammasome activity

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Inflammasomes are large intracellular multiprotein oligomers, which activate the converting enzyme caspase-1 to promote the cleavage and secretion of pro-inflammatory cytokines including interleukin 1β (IL-1β) and interleukin 18 (IL-18) (Kolliputi et al. 2010). The inflammasomes are constructed of different protein subunits, which include the caspase-1/interleukin-1 converting enzyme, PYCARD/ASC caspase recruitment domains, and NALP pattern recognition proteins (Harijith et al. 2014). Once foreign stimuli have been detected by the receptors on these inflammasomes, the proteins that make up the complex can work together as part of the innate immune system to release and activate the precursor forms of the pro-inflammatory cytokines IL-1β and IL-18 (Crane et al. 2014) (Fig. 1). Properly functioning inflammasomes lead to acute systematic inflammation, however, dysfunctional inflammasome activity has been shown to strongly correlate with the pathogenesis of several chronic and destructive diseases, including cancer, colitis, type I diabetes, multiple sclerosis and vitiligo (Lamkanfi et al. 2011). Therefore, it is of vital importance that the complete function of inflammasomes and their signaling pathways be well understood (Wang et al. 2014).

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In a recent issue of *Nature Immunology*, Hara et al. have shed new light on the role of the kinases Syk and Jnk in the activation of the NLRP3 and AIM2 inflammasome complexes. Protein phosphorylation by kinases plays a key role in protein regulation, as it is key in controlling protein activity (Johnson 2009). However, the current relationship between inflammasome activation and phosphoregulation is not yet well understood. To study the role of kinase-mediated inflammasome regulation, macrophage cells were pretreated with Syk and/or Jnk inhibitors prior to stimulation using an activator of NLRP3 and AIM2 inflammasomes. Results showed that IL-18 secretion was significantly lowered following this pretreatment. This effect, however, was only seen in the NLRP3 and AIM2 inflammasomes as the NLRC4 inflammasome showed no significant decrease in IL-18 secretion. Knockout and knockdown of Syk and Jnk kinases in these cells also showed positive results for the decrease of IL-18 secretion in response to stimulation. Based on this finding, the authors suggested that Syk and Jnk kinases play an important role in the activation of NLRP3 and AIM2 inflammasome.

Caspase-1 is an interleukin-converting enzyme that plays a key role in cleaving the precursor forms of IL-1β and IL-18 to their more active forms (Shalini et al. 2014). Hara et al. found that both the use of Syk and Jnk kinase inhibitors and their knockdown using siRNA led to near complete abolishment of caspase-1. The use of other kinase inhibitors, however, did not affect caspase-1 activation. Testing the activity of this enzyme argues against the possibility of these kinase inhibitors and any possibly related nonspecific effects (Platt et al. 2015).

ASC-NLRP3 complexes were observed in macrophage cells, and after pretreatment with an inhibitor of Syk or Jnk, the number of these complexes remained constant. However, following stimulation of the NLRP3 inflammasome, there was a signifi-

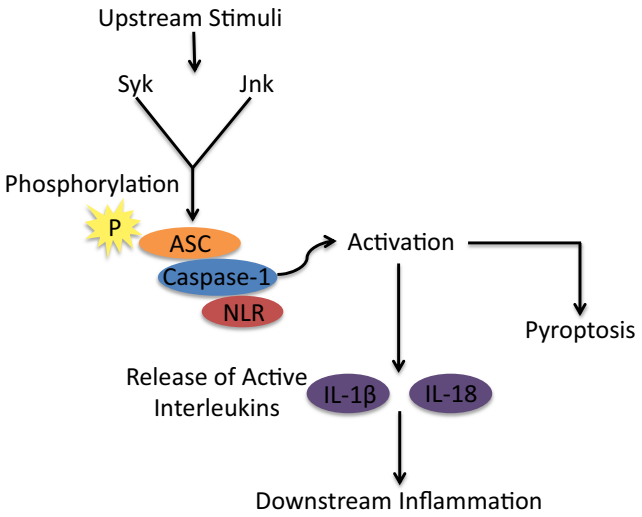

Fig. 1 *General NLRP3 and AIM2 inflammasome activation*. Following a stimulus to the macrophage cell, the inflammasome complex transfers into an active state. The adaptor ASC is then phosphorylated by the inflammatory kinases Syk and Jnk, leading to the activation of pro-caspase 1. Activated pro-caspase 1 leads to the activation of IL-1beta and IL-18, both of which cause downstream inflammation after being detected by other cells. This is a key major process in the general innate immune response pathway

cant difference in the number of ASC speck aggregates that formed. Hara et al. found that unlike the interaction between ASC and NLRP3, the formation of ASC specks required signaling via the Syk and Jnk kinases. These findings by the authors led them to investigate when and where exactly the adaptor ASC was phosphorylated. Data from the in situ proximity ligation assay (PLA) revealed not only that ASC speck complexes were observed in or around the nucleus following stimulation, but also that ASC was only phosphorylated via Syk and Jnk following NLRP3 and AIM2 activation (Hara et al. 2013). The authors suggested that the Tyr144 site was phosphorylated by the Jnk kinase, however, there was insufficient data required to justify that this same site was being phosphorylated by the Syk kinase.

Next, in vivo experimentation was performed using Syk and Jnk deficient chimera mice (Ahn et al. 2014). Injection of monosodium (MSU) or alum into these mice stimulated a strong inflammatory response, as seen in the IL-1β-induced recruitment of inflammatory cells into the peritoneal cavity and included neutrophils, monocytes, and macrophages (Kool et al. 2008). The results indicated that chimeras with deactivated Syk and Jnk kinases showed a decrease in the number of the aforementioned inflammatory cells. In vivo results suggest that Syk and Jnk signaling in MSU/alum-induced mice are required for the activation of the NLRP3 inflammasomes and any related inflammatory responses.

Overall, this study by Hara et al. brings light to a formerly unidentified mechanism of activation for the NLRP3 and AIM2 inflammasomes via the Syk and Jnk kinases. These ASC-containing inflammasomes in macrophages are phosphorylated by way of the Syk and Jnk pathway, contributing to the formation of ASC speck aggregates (Hara et al. 2013). The phosphorylation of the adaptor-ASC is required for the activation of caspase-1 and downstream release of pro-inflammatory cytokines, including IL-18 and IL-1β. Although further investigation are required to determine the precise roles of kinases in inflammasome activation, this phosphoregulatory pathway has emerged as a key controlling mechanism for inflammasomes (De Nardo et al. 2014). It is still largely unclear how Syk and Jnk signaling regulate the formation of ASC specks, and if microtubules play any vital roles in the migration of ASC during speck formation (Misawa et al. 2013). Further mechanistic studies may be required to address these questions, as well as to investigate whether or not any other cellular events may contribute to speck aggregation. As mentioned earlier, it is of vital importance that the whole function of inflammasomes and their signaling pathways be well understood. Current implemented therapies have appeared to be effective in treating several immune diseases stemming from deregulated inflammasome activity, however, compounds designed to specifically inhibit phosphorylation activity of Syk, Jnk and other pro-inflammatory kinases may serve as prime drug candidates for treatment of such diseases (Liao et al. 2013). Since inflammation is prevalent in so many diseases, this study by Hara et al. provides a firm foundation on the development of potentially effective therapeutic treatments against inflammasome triggered auto-inflammatory syndromes.

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