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Cross-regulation between the IL-1β/IL-18 processing inflammasome and other inflammatory cytokines

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Abstract

The inflammasome-forming NLRs are well characterized members of a protein complex mediating the activation of caspase-1 and the cleavage of pro-IL-1β and pro-IL-18 into their active, secreted forms. New data suggest that components of the inflammasome cascade may have roles in influencing inflammasome-independent pathways of cytokine production. These influences on other immune cytokine pathways are complemented by data suggesting that non-inflammasome cytokines can influence the activation of the inflammasome, either directly or by influencing transcription of inflammasome components. The crosstalk between these cytokine cascades may lead to increased abilities for the cell to respond to diverse pathogen threats.

Keywords

Inflammasome; IL-1β; NLR; ASC

Introduction

IL-1β and IL-18 are potent pro-inflammatory cytokines that promote a variety of innate immune processes associated with infection, inflammation, and autoimmunity [1]. A fine balance is required to ensure host defense against viral and bacterial pathogens without tissue damage caused by an over-abundant inflammatory response. Perhaps this is why mammals have developed a two-step system for the regulation of these cytokines. IL-1β and IL-18 are produced as cytosolic precursors and, unlike most secreted proteins, they typically require secondary proteolytic cleavage induced by the inflammasome for activation and secretion. The inflammasome is composed of either a nucleotide-binding domain leucine-rich repeat (NLR) protein such as NLRP1, NLRP3, NLRC4, or NLRC5, the HIN-200 domain-containing protein Aim2, or the cytosolic RNA helicase RIG-I; tethered by the adaptor molecule ASC/Pycard to caspase-1, which provides the enzymatic activity of the complex [1,2]. Assembly of the inflammasome and enzymatic processing of IL-1β and

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IL-18 is activated by a wide range of stimuli, including bacteria, viruses, and danger signals (DAMPs), such as ATP.

Despite the clear role of the inflammasome in IL-1β and IL-18 processing, evidence is emerging to suggest that the function of the inflammasome and its constituents extends beyond these cytokines. For example, *Asc*, *Caspase-1*, and *Nlrp3*-deficient mice each are resistant to septic shock, whereas IL-1β/IL-18 double knockout mice are susceptible [3,4]. Likewise, clearance of flagellated bacteria is dependent on NLRC4 and caspase-1, but is independent of IL-1β and IL-18 [5]. A variety of effector mechanisms for caspase-1 have been proposed that could explain these IL-1β/IL-18- independent activities [6]. The best characterized of these is the ability of caspase-1 to promote pyroptosis independent of other inflammasome components [7]. Additionally, there are several cellular processes and signaling pathways that the inflammasome NLRs and ASC have recently been shown to regulate independent of caspase-1 activation. In the first part of this review, we discuss the emerging role of the inflammasome components in the regulation of non-inflammasome cytokines. Evidence is also emerging to suggest that non-inflammasome cytokines are involved in the regulation of the inflammasome, and this is discussed in the later part of the review. The crosstalk between the inflammasome and non-inflammasome cytokines is likely to provide expanded control of inflammatory processes that have biological consequences for the response to viral and bacterial pathogens and in autoimmunity.

Influence of inflammasome components on the activation of non-Inflammasome cytokines and cytokine signaling pathways (Figure 1)

The emerging role for ASC in the regulation of non-inflammasome cytokines and chemokines through NF-κB and MAP kinase signaling

Recent evidence suggests that ASC has a role in several inflammatory processes independent of its role in caspase-1 activation and IL-1β/IL-18 processing. As an example, ASC is required for a caspase-1-independent form of cell death in response to several pathogens [8–10]. $Asc^{-/-}$ mice are less susceptible to *Mycobacterium tuberculosis*, yet have unimpaired production of IL-1β[11,12]. Furthermore, ASC, but not other inflammasome components, is required for antigen-induced murine arthritis [13,14] and humoral immunity following vaccination [15]. In the latter study, changes in the activation of *Mip1*β and *Mip2* were observed, suggesting that the role of ASC in the activation of cytokines and chemokines may extend beyond IL-1β and IL-18. A more recent study demonstrated that the inflammasome is activated in mice following administration of a high fat diet and that levels of *Tnfa* and *Mcp1* in livers from $Asc^{-/-}$ mice are reduced following administration of insulin, providing further evidence for a role for Asc extends beyond the processing of IL-1β and IL-18 [16].

Recent studies have revealed a possible mechanism for ASC-dependent activation of non-inflammasome cytokines and chemokines. ASC is required for the activation of an array of inflammatory cytokines in monocytic cell lines following infection with *Porphyromonas gingivalis*, including TNF-α, IL-6, IL-8 and IL-10 [17]. The activation of these cytokines was independent of IL-1R signaling and caspase-1 activation, but correlated with the activation of NF-κB. NF-κB activation by ASC has also been verified in 293T cells by genetic reconstitution [18–20], while the role of ASC in NF-κB activation in mouse macrophages is less clear. A recent study has expanded the list of ASC-dependent cytokines in human and mouse macrophages to include a panel of chemokines whose expression is controlled by the MAP kinases ERK and JNK [21]. MAP kinase activity was shown to be regulated by the dual-specificity phosphatase DUSP10/MKP5 and be independent of the inflammasome. ASC has also recently been shown to activate AP1 signaling in a

reconstituted cell system and to transcriptionally induce TNF, IL-6, IL-8 and CXCL2. Activation of AP1 required MAP kinase activation and caspase-8 signaling, but was independent of caspase-1 [22]. Collectively, these studies suggest that ASC may mediate the activation NF-κB, MAP kinase, and caspase-8 signaling pathways to transcriptionally activate non-inflammasome cytokines and chemokines.

Potential roles for the inflammasome NLRs, RIG-I and Aim2 in the regulation of non-inflammasome cytokines and chemokines

There is evidence to indicate that the inflammasome NLRs may have functions independent of the inflammasome as well. In a renal ischemia-reperfusion injury model, the absence of *Nlrp3*, but not *Asc, Il1β* or *Il18*, was sufficient to protect mice [23]. Many of the noninflammasome NLRs (e.g., NOD1, NOD2 and NLRP12) have defined roles in the NF-κB and MAP kinase pathways [24,25]; however, it is unknown whether the inflammasome NLRs have similar effects. The possible exception is NLRC5, an additional member of the NLR protein family that has recently been identified as a potential inflammasome protein [26,27]. NLRC5 binds to IKKα and IKKβ and blocks signaling dependent on NF-κB, including the production of TNF-α and IL-6 [28]. This role of NLRC5 in influencing NF-κB signaling is controversial and has been confirmed in some studies [29] but not others [27,30,31]. A mechanism for NLRC5 in influencing interferon responses has also been suggested. NLRC5 binds RIG-I and MDA5 to inhibit type-I interferon responses, analogous to the role of the non-inflammasome protein NLRX1 [25,28]. Other studies have indicated that overexpression of NLRC5 can lead to the induction of IFN-γ-dependent signaling pathways, including STAT1 phosphorylation, and that knockdown of NLRC5 can abrogate Sendai virus and poly (I:C)-induced type-I interferon responses [30,31]. Once again, these results are confounded by conflicting results from other studies [27,29]. The use of different cell types in these studies make it difficult to directly compare results; however, it is possible that this novel NLR protein plays an important role in the regulation of both inflammasome and non-inflammasome cytokines and may provide insight into the mechanisms by which these pathways intersect. The identification of specific NLRC5-dependent ligands will likely aid in elucidating its role.

Additional studies show cross-regulation between inflammasome activation and non-inflammasome cytokines in the viral response. Cytosolic viral RNA is known to activate a complex containing RIG-I and MAVS that induces both IRF-mediated transcription of type I interferon and NF-κB-mediated transcription of *IL-6* and *TNFα*. Recently, RIG-I was also shown to bind ASC in a caspase-1-activating inflammasome that processes IL-1β [32]. Likewise, the viral DNA inflammasome sensor Aim2 has been suggested in some studies [33,34], but not others [35,36] to have a secondary role in the regulation of NF-κB and the transcription of IFN-β. Thus, RIG-I and Aim2 may both serve dual roles as viral inflammasome sensors and activators of non-inflammasome cytokines.

Role for the inflammasome in the secretion of leaderless proteins other than IL-1β and IL-18

Most secreted proteins contain signal peptides that mediate targeting to the endoplasmic reticulum in the classical secretory pathway. The proteins are then transported through the Golgi apparatus, where Golgi-derived secretory vesicles fuse with the plasma membrane for release into the extracellular space. More than 20 proteins in addition to IL-1β and IL-18 are known to be released by unconventional protein secretion independently of the ER and Golgi. The list includes signaling molecules involved in inflammatory, cell survival, and repair responses, such as HMGB1, IL-1α, galectins 1 and 3, and FGF2. Though these proteins are not thought to be direct substrates for caspase-1 cleavage, recent studies suggest that caspase-1 is required for their secretion [37]. *Nlrp3* and *Asc* also have a demonstrated

role in the LPS-driven release of IL-1α [38,39] and HMGB1 [3]. Several additional non-cytokine substrates for caspase-1 have been identified including caspase-7 [40,41], and it is possible that one of these substrates may mediate the processing of these leaderless cytokines. Alternatively, several trafficking proteins have been identified as caspase-1 substrates, including Rac2, Rab GDI, Rho RDI beta, and RAB7 [40]. A recent study has also identified Rab39a as a caspase-1 binding partner that is involved in the secretion of IL-1β [42], and it is possible that Rab39a or one of these other trafficking proteins may regulate the secretion of non-inflammasome cytokines by the unconventional secretory pathway.

Influence of non-inflammasome cytokines and cytokine signaling pathways on the inflammasome (Figure 2)

Effects of TNF-α, MAP kinase and NF-κB on the inflammasome

The canonical model of inflammasome activation involves “Signal 1”, transcriptional upregulation of *pro-IL1b* and *pro-IL18* often induced by TLR stimulation, followed by “Signal 2”, caspase-1-mediated cleavage of pro-IL-1β and pro-IL-18 into their mature forms (reviewed in[1]). Early data indicated that TNF-α could induce IL-1β secretion [43]. More recently it has been shown that TNF-α, and to a lesser extent IL-1α and IL-1β itself, could induce caspase-1 activation and IL-1β secretion [44]. These data indicate that other cytokines may be able to substitute for a TLR-mediated stimulus to induce “Signal 1”. This TNF-α-mediated caspase-1 activation was shown to require translation and NF-κB activation. However, unlike LPS pretreatment, TNF-α pretreatment resulted in sustained ATP-dependent IL-1β secretion by the NLRP3 inflammasome, implying that inflammasome induction by cytokines may amplify an inflammatory response and differ in the quality of inflammasome activation. Additionally, the use of pharmacological inhibitors has indicated that TLR-mediated signaling through TAK1 may influence the activity of the inflammasome in a transcription-independent manner [45].

It is likely that additional cytokines will be shown to induce signal 1 in inflammasome activation. The human monocyte-derived cell line THP-1 is routinely used in studies of the inflammasome following PMA maturation, which leads to a strong increase in the transcription of pro-*IL1b* [46], suggesting that MAP kinase activation may lead to signal 1. Other studies have indicated that NF-κB stimulation via RANKL or stimulation via PMA or IFN-γ cannot lead to signal 1 in mouse macrophages [44], meaning that this may be an instance of differential control of the inflammasome in different cell types.

In addition, early studies have shown that the expression of *NLRP3* is induced by TNF-α in human monocytes, indicating another way in which cytokines can amplify inflammasome activation [47]. Like the expression of *pro-Il1b*, the expression of *Nlrp3* has also been found to be dependent on NF-κB activation [48]. In fact, the expression of *Nlrp3* was influenced by many of the same stimuli that induce the expression of *pro-IL1b*, indicating that these stimuli may be leading to more amplification than previously appreciated [49]. Influenza virus infection has also been shown to induce *Nlrp3* transcription in mouse airway epithelial cells and total lung homogenates [50]. Analysis of the *NLRP3* promoter revealed the presence of Sp1, c-Myb, AP-1, and c-Ets sites, indicating that the regulation of this sensor is likely complex [51]. *NLRC5* has also been shown to be transcriptionally regulated by NF-κB [28]. Further, Syk has been shown to enhance inflammasome activation by binding Asc following phosphorylation by Lyn in response to malarial hemozoin [52] or by influencing *pro-IL1b* transcription in response to *Candida albicans* [53]. The transcriptional regulation of inflammasome components likely represents an area of convergence of many proinflammatory pathways.

Effects of interferon on the inflammasome

Increasing evidence suggests that interferon signaling can also influence inflammasome activation. Type-I interferon levels can enhance Aim2 protein levels, while deficiency in the interferon receptor or STING signaling pathways reduces the activity of this inflammasome [34,35,46,54]. Other studies have indicated that IFN-β's role in enhancing the activity of the Aim2 inflammasome is not dependent on changes in *Aim2* transcription and depends on influencing the accessibility of Aim2 ligands [36]. In addition, transcription of the novel inflammasome member *NLRC5* has been shown to be regulated by IFN-γ and IFN-γ-dependent infection and its promoter contains two predicted STAT binding sites [29,30,55]. Another study suggested that *NLRC5* is transcriptionally regulated by two stimuli of type-I interferon, poly (I:C) and Sendai virus [31]. It will be interesting to determine whether NLRC5 senses pathogens that are also strong inducers of interferon.

An intriguing study also highlights the potential for direct interaction between the interferon and inflammasome pathways. Pretreatment with IFN-β was shown to inhibit the activation of caspase-1 downstream of stimulation with NLRP1 and NLRP3 agonists and exacerbate infection with the NLRP3-dependent pathogen *Candida albicans* [56]. These data are particularly interesting as they provide an explanation for the immunosuppression observed following viral infection. In addition, this study showed that monocytes from patients undergoing IFN-β treatment produced reduced levels of IL-1β, providing a mechanism for the utility of IFN-β as an MS treatment [56]. IFN-γ has also been shown to enhance the ability of macrophages to produce IL-1β, although the mechanism is unknown [57].

Conclusions

Our original understanding of the inflammasome was as a platform for the activation of caspase-1 and cleavage of the pro-forms of IL-1β and IL-18 into active cytokines. Emerging data indicates that the components of the inflammasome cascade can have roles in pathways independent of their roles in the inflammasome. New data is highlighting potential roles for other inflammatory cytokines in influencing inflammasome activation via transcription and mechanisms other than transcription as well. While the study of pro-inflammatory cytokines has been bifurcated into examination of either the interferon pathway or inflammasome-dependent IL-1β secretion, it is now evident that substantial crosstalk exists between these pathways. Likewise, the members of the NLR family were originally thought of as either inflammasome-forming NLRs or non-inflammasome NLRs. While it is still true that the primary function of the inflammasome NLRs is the formation of the inflammasome complex for IL-1β and IL-18 secretion, many of these NLRs serve functions similar to those described for non-inflammasome NLRs. In fact, these proteins may be functioning as parts of different multicomponent signalosomes depending on cell type and other NLR proteins present and may not be restricted to acting in only one of these signalosomes. This type of response may allow multiple signals from diverse pathogen-sensing pathways to be integrated and allow the cell to generate an appropriate response. It is likely that the data presented in this review are only the first observations of this sort of crosstalk between inflammasome components and other cytokine signaling pathways and that many more will be described in the future.

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* Of special interest

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Highlights

- The IL-1β/IL-18-processing inflammasome is composed of an NLR sensor, Aim2 or RIG-I; the adaptor ASC/Pycard; and caspase-1.
- Recent evidence suggests that many of these inflammasome components have secondary roles in the transcriptional induction of non-inflammasome cytokines through the activation of NF-κB and MAP kinase signaling pathways.
- Caspase-1 also is proposed to have a role in the processing of growth factors through the unconventional secretory pathway.
- A variety of studies also suggest that non-inflammasome cytokines may influence the activation of the inflammasome through transcriptional induction of inflammasome components and direct interaction with inflammasome components.
- The cross-regulation between these inflammatory cytokine cascades may lead to increased abilities for the cell to respond appropriately to diverse pathogen threats.

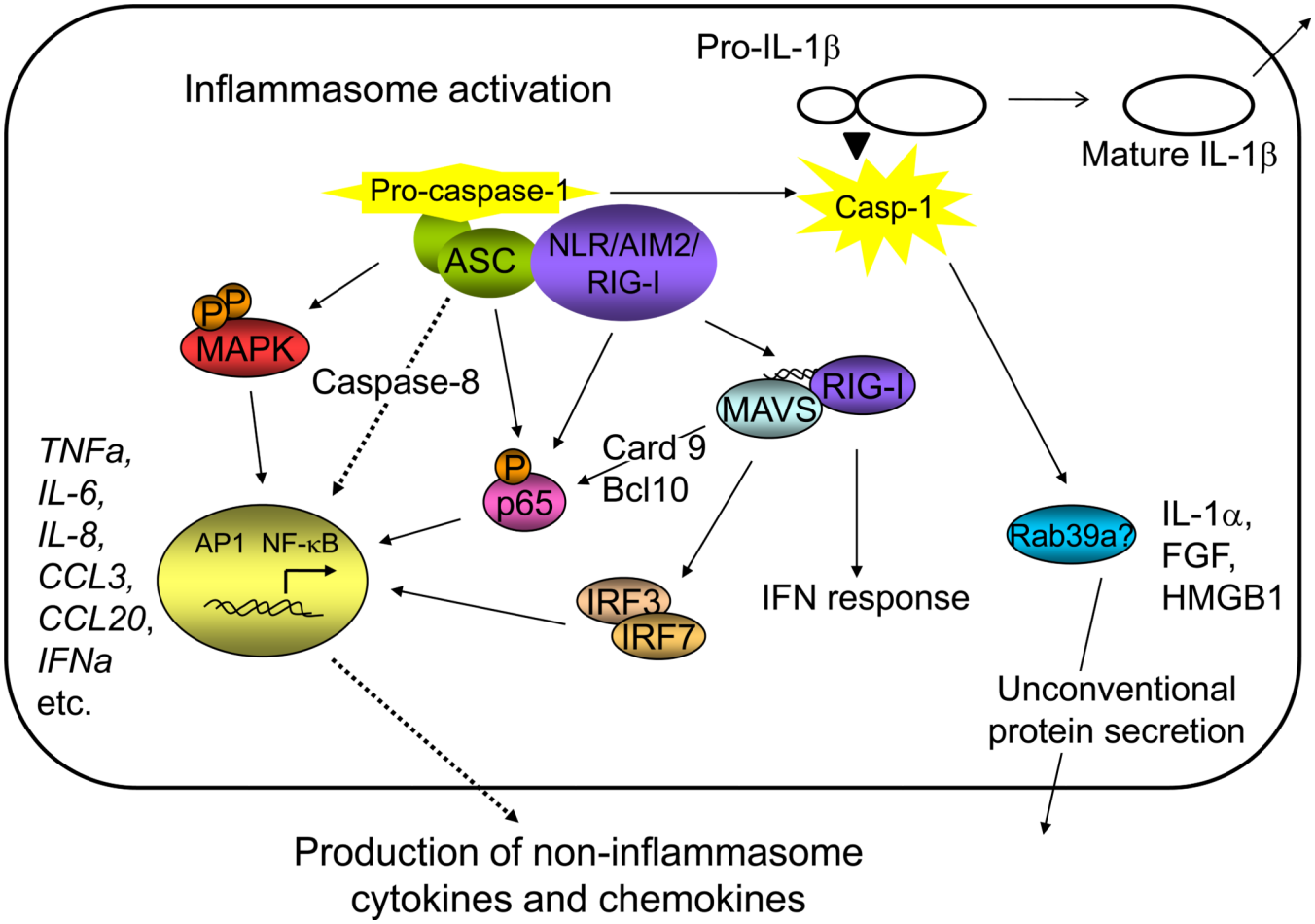

Figure 1. Influence of inflammasome components on the activation of non-Inflammasome cytokines and cytokine signaling pathways

Recent evidence suggests that ASC has an inflammasome-independent role in the activation of MAP kinase and NF-κB signaling, which leads to the transcriptional activation of a panel of cytokines and chemokines. Inflammasome NLRs, including NLRP3 and NLRC5, and the inflammasome viral DNA sensors AIM2 and RIG-I have also been shown to influence the activation of NF-κB and subsequent activation of cytokine transcription. Additional evidence suggests that AIM2 may directly affect the induction of IFN-α by viruses through interaction with the RIG-I/MAVS complex. Recent evidence also suggests that caspase-1 may influence the production of additional cytokines that are processed through the unconventional secretory pathway.

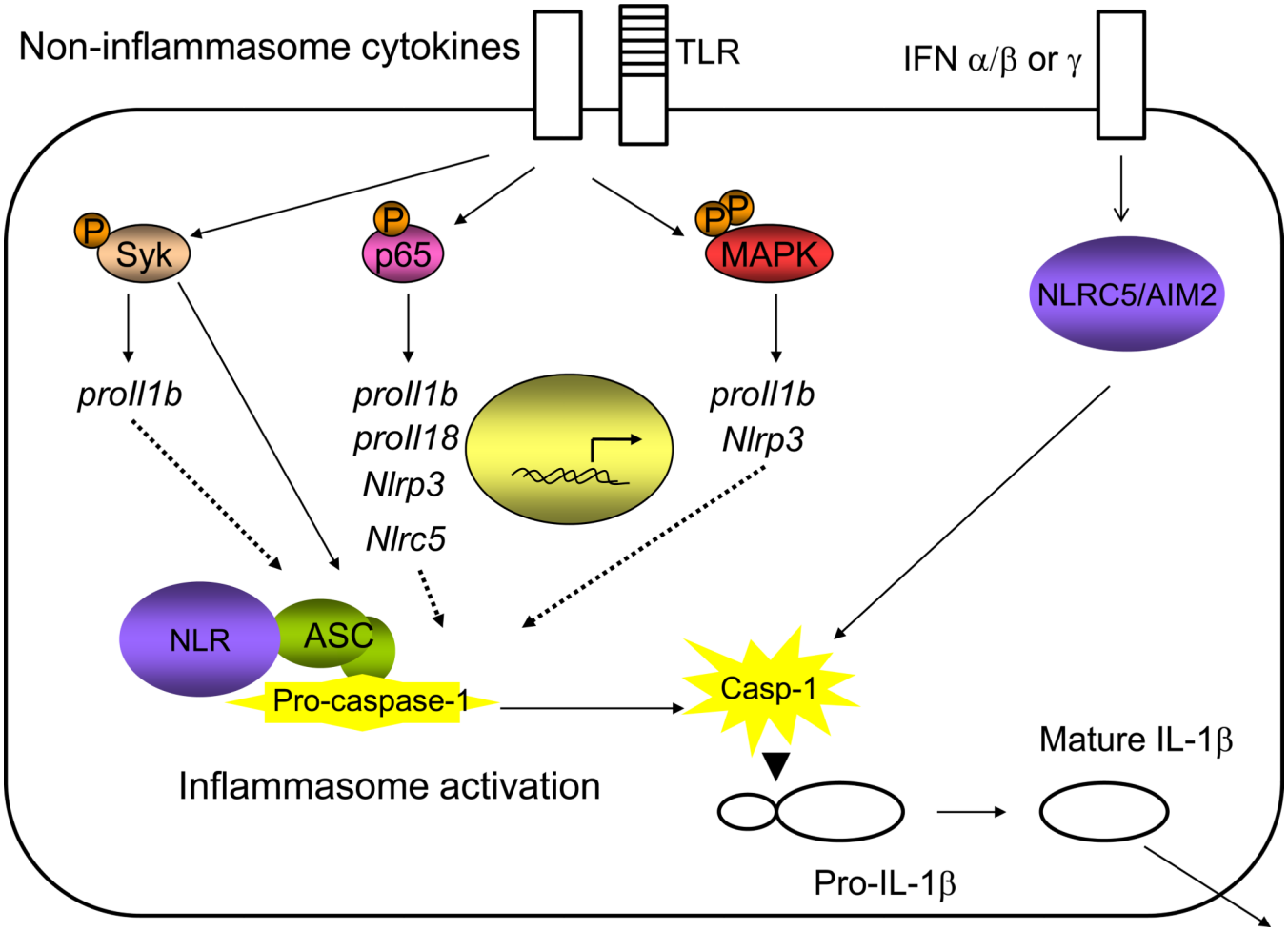

Figure 2. Influence of non-inflammasome cytokines and cytokine signaling pathways on the inflammasome

Many immune pathways downstream of non-inflammasome cytokines influence inflammasome activation, either by transcriptional upregulation of inflammasome components or direct interaction with the inflammasome. While the understanding of the transcriptional control of the NLR sensors is not well understood, it has been demonstrated that NF-κB signaling, Src family kinases, Syk kinase, MAP kinase, and interferon signaling are involved in NLR transcription. Syk kinase and molecules in the interferon and NF-κB signaling cascades can also interact with inflammasome components to influence inflammasome activation.