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RESEARCH HIGHLIGHT

PKR stirs up inflammasomes

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Inflammasomes are multiprotein complexes that detect and respond to foreign and endogenous danger signals by activating caspase-1; active caspase-1, in turn, matures the pro-inflammatory IL-1β family cytokines by cleaving their pro-forms into the biologically active cytokines. The upstream mechanisms leading to inflammasome activation, in particular for the NRLP3 inflammasome, remain poorly understood. Lu and colleagues identify a new function of Protein Kinase R (PKR) for activating the NLRP1, NLRP3, NLRC4 and AIM2 inflammasomes, thus identifying a potential new target for treating inflammasome-mediated diseases.

Every day, our bodies are challenged with microbes and endogenous danger signals that have the potential to cause inflammation, tissue dysfunction and disease. To identify and respond to these numerous potential dangers, our immune system evolved a range of innate signaling receptors. Among these, the inflammasomes recognize molecular signatures in the cytosol that are signs of infections or cell stress. For example, the AIM2 inflammasome assembles upon the presence of double-stranded DNA (dsDNA) in the cytosol and the NLRP3 inflammasome can be activated by extracellular danger signals, such as aggregated or crystalline substances, which can cause harm to cellular membranes. Once assembled, the inflammasomes act as platforms to trigger the activation of IL-1β cytokines, which then orchestrate inflammatory responses that guide immune cells to the site of disturbance and initiate crucial immune effector mechanisms [1]. Caspase-1 further sets free so-called alarmin molecules, such as HMGB1, which contribute to the inflammatory response. HMGB1 is thought to be part of many inflammatory conditions, and has already been the target of several therapeutics for immune diseases [2]. Given the vigorous pro-inflammatory response set in motion after inflammasome activation, a tight regulation of inflammasomes and caspase-1 activation is critical to prevent collateral damage to host tissues. Expectedly, aberrant activity of inflammasomes can lead to metabolic, inflammatory and autoimmune diseases [3]. While our knowledge about how inflammasomes contribute to inflammation has rapidly increased in recent years, much mystery still surrounds the molecular details of their activation.

Recent findings have uncovered another piece to the puzzle, showing that PKR is involved in the activation of the NLRP1, NLRP3, NLRC4 and the AIM2 inflammasomes [4]. This new link is, at first sight, quite surprising for a protein that primarily functions as an antiviral protein. PKR binds dsDNA, upon which it autophosphorylates, then activates the translation initiation factor EIF2α, halting translation and aiding the cell in viral clearance [5]. Further evidence for its general antiviral effects comes from recent studies that have identified that PKR constitutively inhibits actin severing activity by blocking gelsolin. Upon PKR activation gelsolin is liberated and aids in the defence against viral infection by controlling cellular virus uptake [6]. PKR can also exert signaling functions, which, however, are less well characterized. Many stimulants that promote inflammasome activity are also known to be stimulators of PKR [7-10], which raised the question of whether the activation of PKR and inflammasomes are connected. The study by Lu and colleagues has indicated that this is indeed the case. They discovered that peritoneal macrophages from mice lacking the kinase domain of PKR released lower levels of HMGB1 and IL-1β cytokines compared to wild-type macrophages in response to stimulation with double-stranded DNA or RNA, crystals, anthrax lethal toxin or certain bacteria [4]. Furthermore, consistent with a role of PKR in inflammasome activation, cells lacking the PKR kinase domain were partially protected from pyroptosis, a caspase-1-dependent form of cell death [4, 11]. Strikingly, the *in vivo* response of PKR kinase domain deficient mice towards live *E. coli* was also blunted when compared to wild-type mice. PKR kinase domain deficient mice had diminished levels of IL-1β cytokines or HMGB1 and showed reduced neutrophil infiltration to the peritoneal cavity, indicating compromised inflammasome activation. Collectively, this evidence suggests that PKR regulates multiple inflammasomes and PKR could thus be central to the immune response towards microbes and during the development of sterile inflammation [4]. As the different inflammasomes are engaged by structurally diverse activators and are most likely activated by different upstream processes, this broad function of PKR raises the question, by which mechanisms PKR influences the activity of these inflammasomes. The PKR-targeted inflammasomes, NLRP1, NLRP3, NLRC4 and AIM2 all require the adap-

tor molecule ASC and the downstream effector molecule caspase-1 for the activation of IL-1β cytokines. Given that other endogenous regulators and various viral proteins target these shared signaling molecules, an effect of PKR downstream of inflammasomes would have seemed to be most logical and could have easily explained the broad effect of PKR on inflammasome activity. Surprisingly, however, judging from a series of elegant and technically challenging experiments the authors come to different conclusions.

To further elucidate the potential mechanisms of inflammasome inhibition, reconstitution assays and immunoprecipitation assays were performed. When the components of the NLRP3 inflammasome were overexpressed in HEK cells, it was found that PKR could drive effective NLRP3 inflammasome reconstitution and, conversely, RNAi-mediated reduction of PKR reduced the experimental NLRP3 reconstitution and activation. Moreover, PKR and NLRP3 co-immunoprecipitated from macrophage cell lysates and stimulation with either polyI:C or ATP further induced NLRP3/PKR complex formation and increased caspase-1 activation [4]. Most surprisingly, PKR was shown to interact with all individual functional domains of NLRP3 (PYD, NACHT and LRR) as well as with NLRP1, NLRC4 or AIM2. This wide scope of protein/protein interactions raises, of course, the question of specificity and opens the possibility that the interaction between PKR and inflammasome sensors could be mediated via intermediate proteins [4]. Importantly in this context, PKR activity was found to be required for promoting caspase-1 activity in a cell-free reconstitution system, suggesting a direct intimate connection with one of the assembled proteins. Gel filtration and native gel analysis of the NLRP3 inflammasome complex further revealed that PKR co-migrated with the active, high molecular weight inflammasome fractions, arguing that PKR is an integral part of the activated inflammasome complex. As the primary role of PKR is to phosphorylate target proteins, an intriguing mechanism would be that PKR could license inflammasome activation via phosphorylating inflammasome proteins. However, no such evidence could be demonstrated by the authors.

The new findings by Lu and colleagues have helped to further understand how inflammasomes are regulated (Figure 1). By fitting a new piece into the puzzle, they have provided a potential new therapeutic target for inflammasome-mediated disease. Further work is now needed to better understand the molecular mechanisms by which PKR can exert such a broad regulatory role. In addition, PKR's regulatory function for inflammasomes needs to be corroborated in mice that lack the full protein as one cannot exclude potential influences of the DNA binding domain that could have functions in mice lacking only the kinase domain of PKR. It would also be interesting to test whether PKR's function in the regulation of gelsolin-mediated cytoskeletal control has an impact on inflammasome activation [5]. This is particularly attractive as the gelsolin family member, flightless, has previously been shown to control inflammasome activation by way of directly blocking caspase-1 and caspase-11 and by modulating their subcellular localization [12]. We are excited to learn more about PKRs' ever expanding capacity to control innate immune responses.

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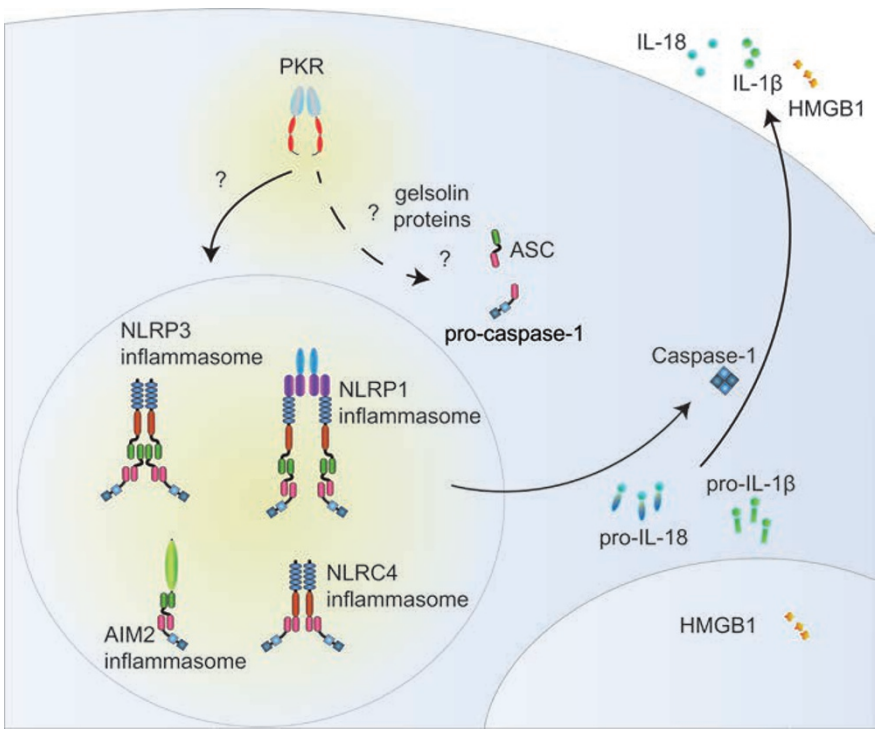

Figure 1 A summary of the newly proposed inflammasome activation pathway including PKRs' recently found role. The activation of several inflammasomes results in the release of pro-inflammatory cytokines and alarmins including IL-1β, IL-18 and HMGB1. PKR was shown to interact with the NLRP1, NLRP3, NLRC4 and AIM2 inflammasomes, however, the mechanisms of inflammasome regulation remain elusive. Several questions remain unanswered, such as whether PKR modulates downstream inflammasome signaling, by interacting with the common adaptor protein ASC or pro-caspase-1.

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