

PKR as a Regulator of Inflammasome Activation

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Detection of pathogen by pattern recognition receptors leads to activation of inflammasome which plays a crucial role in immune system. The inflammasome regulates the release of cytokines, such as interleukin (IL)-1 β , IL-18 and high-mobility group box 1 (HMGB1). Double-stranded RNA-dependent protein kinase (PKR) is a critical component of an inflammatory complex. Recently, the critical role of PKR was reported in regulation of multiple inflammasomes.

Key Words: Double-stranded RNA-dependent protein kinase, High-mobility group box 1, Inflammasome, Nod-like receptor family pyrin domain containing 3

In Nature on 30th August 2012, Lu *et al* reported the novel role of double-stranded RNA-dependent protein kinase (PKR) in inflammasome activation and high-mobility group box 1 (HMGB1) release (1). Mammalian immune system evolved, over a period of time, a range of innate signaling receptors to respond the endogenous as well as exogenous potential dangers. Toll-like receptors detect extracellular or vacuolar stimuli while nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) recognize molecular signatures in the cytosol (2). Nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) play important role in immune responses, by regulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and inflammasome signaling. The activated inflammasome serves as platform for caspase-1 activation which results in proteolytic processing and release of mature IL-1 β , IL-18, and initiates onset of pyroptosis. Pyroptosis is proinflammatory form of pro-

grammed cell death (3). HMGB1 is nonhistone nuclear protein found in nucleus and cytoplasm of almost all cell types. HMGB1 can be secreted in response to diverse stimuli including cytokines, polyinosinic-polycytidylic acid poly(I:C), pathogen-associated molecular patterns and some cellular stress (4, 5). HMGB1 is actively secreted by innate immune cells and its level in tissue or serum is increased during infection. Activation of macrophage by lipopolysaccharide, tumor necrosis factor- α and poly(I:C) results in acetylation of HMGB1 and leads its translocation from nucleus to cytoplasm for further action (6).

Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome has been shown to play role in many human diseases including obesity, type 2 diabetes, gout, sepsis and, cardiovascular diseases (7). Monosodium urate, external ATP and silica are the known activators of NLRP3 inflammasome and known as canonical stimuli. Poly(I:C) is an immunostimulant which is structurally

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similar to double stranded RNA and known to stimulates TLR3. PKR is an antiviral protein which is activated by double stranded RNA. Binding of PKR to its ligand results in its autophosphorylation that in turn activates translation initiation factor eIF2 α , which prevents viral protein synthesis (2). Here we summarize the results of Lu *et al* that PKR interacts with various inflammasome components and broadly regulates inflammasome activation (1).

Lu *et al* generated PKR-deficient (*Pkr*^{-/-}) mice by genetic deletion and compared HMGB1 secretion with wild type. Stimulation with poly(I:C) resulted significantly lower secretion of HMGB1 in *Pkr*^{-/-} macrophage as compared to wild type (*Pkr*^{+/+}) macrophages. Similar results were observed by pharmacological inhibition of PKR in *Pkr*^{+/+} macrophages in dose-dependent manner (1). Phosphorylation of PKR and release of HMGB1 in *Pkr*^{+/+} macrophages showed similar pattern in response to other danger signals, including ATP, monosodium urate, adjuvat aluminium, and live *E. coli*. Activation of inflammasome in macrophage leads to programmed inflammatory cell death, termed as pyroptosis. Lactate dehydrogenase is a marker for pyroptosis and genetic deletion of PKR significantly inhibited its release (1). Taken together, these results suggest that release of HMGB1 and inflammasome activation, are regulated by PKR.

Authors further extended their work to identify the role of PKR in the activation of NLRP3 inflammasome and measured caspase-1 activation and cleavage of IL-1 β from *Pkr*^{-/-} and *Pkr*^{+/+} macrophages stimulated by canonical stimuli. As expected caspase-1 activation and cleavage of IL-1 β were significantly inhibited in *Pkr*^{-/-} macrophages. In agreement, similar results were observed in transfection assay of bone-marrow derived dendritic cells with poly(I:C) or *E. coli* RNA. Taken together, these results suggest that PKR has a crucial role in activation of NLRP3 inflammasome (1). For further verification of the results, *in vivo* study was conducted using *Pkr*^{-/-} or *Pkr*^{+/+} mice and challenged with live *E. coli*, to activate NLRP3 inflammasome. In agreement to *in vitro* study, serum level of IL-1 β , IL-18 and HMGB1 were significantly reduced in *Pkr*^{-/-} mice suggesting the role of PKR in regulating inflammasome

dependent cytokine release (1).

HEK293A cells do not normally express NLRP3 inflammasome components. By overexpression of PKR and NLRP3 inflammasome components in HEK293A cells, the study showed the role of PKR in caspase-1 activation and IL-1 β cleavage. In the absence of PKR no caspase-1 activation was observed (1). In a cell free system, NLRP3 inflammasome was reconstituted by combining NLRP3, ASC, pro-caspase-1 and PKR. Addition of poly(I:C) or ATP significantly increased caspase-1 activity but in the absence of PKR there was no caspase-1 activity. In addition, further role of PKR was confirmed by using mutant PKR which failed to activate NLRP3 inflammasome (1). Taken together, these data indicate that PKR physically interacts with NLRP3 and is critical for activation of inflammasome (1). Stimulation of macrophages with anthrax lethal toxin, poly (dA:dT) or *Salmonella typhimurium* results in activation of NLRP1, AIM2 and NLRC4 inflammasome respectively (1). This significantly enhanced the autophosphorylation of PKR. By overexpression studies of PKR in HEK293A cells they showed that PKR regulates NLRP1, AIM2 and NLRC4 inflammasome signaling (1).

Lu *et al* showed that PKR interacts with inflammasome components and plays crucial role in activation of caspase-1, and IL-1 β , IL-18 and HMGB1 release. The molecular mechanism by which PKR plays broad regulatory role, needs to be studied in detail. In future it could be possible to pharmacologically target PKR for treating inflammasome related inflammatory diseases.

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