Role of NOD-like receptor, caspase-1, and pyroptosis in clearance of *Salmonella typhimurium*

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Abstract

Innate immune system plays a critical role in early detection of pathogen and inflammation-associated diseases. Detection of pathogen-associated molecular patterns (PAMPs) by pathogen recognition receptors (PRRs) triggers the activation of inflammatory responses by the immune cells. Macrophage plays a critical role in innate immunity. Detection of pathogen by macrophages leads to activation of innate and adaptive immune response for neutralization and clearance of the pathogen. *Salmonella typhimurium*, an intracellular bacterium can infect and multiply in the cytosol of macrophages. NLR family CARD domain-containing protein 4 (NLRC4), a cytosolic PRR, has a vital role in detection of the infection. NLRC4 can detect flagellin of S. *typhimurium* which results in its clearance by pyroptosis. Clearance of S. *typhimurium* via pyroptosis is dependent on NLRC4- mediated activation of caspase-1, and independent of Interlukin-1β (IL-1β) and IL-18. NLRC4 dependant activation of caspase-1 provides protection against a large number of translocated virulance factors. This method of innate immune detection permits the macrophage to discriminate virulent from avirulent bacteria. In this review we discuss that how *S. typhimurium* is detected and cleared by the innate immune system. (J Med Life Sci 2011;8:21-24)

Key Words : NOD-like receptor, caspase-1, pyroptosis, *Salmonella typhimurium*

Introduction

The innate immune system is characterized by pathogen recognition receptors (PRRs) which include Toll-like receptors (TLRs), retinoic acid-inducible gene (RIG)-1-like receptors (RLRs), NOD-like receptors (NLRs) and C-type lectin receptors (CLRs). PRRs detect pathogen-associated molecular patterns (PAMPs) and activate downstream signaling pathways[1-2]. Macrophage uses two steps detection system to discriminate between pathogenic and non-pathogenic microorganisms. TLRs detect extracellular stimuli which results in downstream immune response triggering transcription, translation and release of specific cytokines including production of precursors of IL-1β and IL-18. NOD-like receptors (NLRs) sense cytosolic stimuli resulting activation of caspase-1 which further regulate proteolytic processing of pro-IL-1β and pro-IL-18[3].

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Delivery of flagellin to cytosol by type three secretion system

Many Gram negative pathogenic bacteria of plants and animals have specialized protein secretion system, known as type 3 secretion system (T3SS)[4], which helps in their engulfment and further modulation of host cell signaling pathways. T3SS is comprised of a bacterial nanoinjector resembling a syringe with a needle- to inject virulent effector proteins to target cells[5]. *S. typhimurium* requires *Salmonella* pathogenecity island-1 (SPI-1) type three secretion system (T3SS) for invasion of host cells[6-7]. PrgJ encodes an essential part of T3SS and is called as rod protein. PrgJ expression results in detection of *S. typhimurium* by macrophages. *S. typhimurium* using SPI1 T3SS, usually infect the cells which do not have NLRC4[3]. Once *S. typhimurium* is inside the cell, it can grow easily in a protected environment. *S. typhimurium* does not express SPI1 or flagellin during the systemic phase of infection rather it expresses a different T3SS, SPI2, which promotes replication in macrophages[8]. The SPI2 T3SS secrets a rod protein called SsaI which is not detected by NLRC4[3]. However, macrophages can detect flagellin or PrgJ rod protein *in vitro*[3, 7]. Rod protein PrgJ and flagellin share amino acid motif which are critical for caspase-1 activation.

Flagellin, PrgJ and PrgI polymerizes in to hollow tube structure that is involved in the formation flagellar filament, rod and needle of SPI1 T3SS respectively. Delivery of PrgJ but not PrgI to cytosol of macrophage can activate NLRC4 and further processing of inflammtory cytokines. T3SS rod proteins are broadly detected by NLRC4 as PrgJ horologes are found in most of the T3SS such as BsaK *(Burkholderia pseudomallei)*, MxiI *(Shigella flexneri)*, PscI *(Pseudomonas aeruginosa)* and EprJ *(enterohemorrhagic Escherichiacoli, EHEC)*. All of these rod proteins share varying sequence similarities which are critical for NLRC4 activation[9].

Role of NLRC4 in detection of cytosolic flagellin

Nucleotide-binding domain leucine-rich repeat containing (NLR) are defined by their tripartite domain structural design, which contains a variable C terminus, a middle NACHT (NAIP, CIITA, HET-E and TP1) domain and a leucine rich repeat domain[10]. Cytosolic flagellin or PrgJ is detected by LRR and N-terminal activates capase-1[3].

An inflammasome is a multiprotein oligomer composed of caspase-1, NLRC4, ASC and sometimes caspase-5. However the exact composition of inflammasome depends upon the stimulator. Here, in case of *S. typhimurium*, inflammasome consists of NLRC4/Ipaf and caspase-1. *S. typhimurium* accidently translocates flagellin and PrgJ to host cell by SPI1 T3SS. Stimulation of NLRC4 in macrophages requires a functional *Salmonella* pathogenicity island 1 type III secretion system[6]. Cytosolic flagellin activates caspase-1 via NLRC4 and is independent of TLR 5 which is required for extracellular flagellin detection[11-12]. Extracellular flagellin is detected by TLR5 which down regulates the signal *via* My-D88 dependant mechanism and at the end results in expression of inflammatory response. This inflammatory response includes expression of proforms of IL-1β, IL-18 as well as release of IL-12 and IL-6[3].

It is exactly not clear that whether ligand directly interacts with receptor part of inflammasome, however, there is an indication that some proteins can function in binding the ligands. The primary function of inflammasome is to control the activation of caspase-1. The activated caspase-1 is involved in pyroptosis, proteolytic maturation and release of pro IL-1-β as well as IL-18. Absent in melanoma 2 (AIM2) lacks the typical NACHT domain of the NLR inflammasome and can form the inflammsome together with ASC. The composition of inflammasome depends upon the stimulus[13].

Caspase-1 activation is dependent upon NLRC4 detection of flagellin or PrgJ

Caspase-1 is a member of cystine proteases, produced as zymogen that is cleaved into 20 kDa (p20) and 10 kDa (p10) subunits[14]. In cytosol caspases exist in inactive proforms and are cleaved by other caspases[15]. The caspase family of proteases is divided into pro-apoptotic (Caspase-2, 3, 6, 7, 8, 9, 10) and pro-inflammatory (Caspase-1, 4, 5, 12) members[16]. NLRC4 activated caspase-1 plays an important role in pyroptosis as well as in proteolytic processing and release of inflammatory cytokines such as Interleukin-1β (IL-1β) and IL-18[15, 17]. The CARD of NLRC4 directly interacts with the CARD of caspase-1 and the activated caspase-1 results in pyroptosis[3]. Although caspase-1 knockout mice are more susceptible to infection with *S. tiphimurium* than wild type mice. Nlrp3 knockout or ASC knockout mice are not[18], which suggests that multiple pathways may lead to caspase-1 activation in response to *S. tiphimurium*.

Apoptosis-associated speck-like protein containing a CARD or ASC is an adaptor protein and it contains a pyrin domain as well as a caspase-recruitment domain (CARD). ASC plays a role in bridging the pyrin domain of NLRP3 to the card of Caspase-1[19]. In clearance of *S. typhimurium*, there is no role of ASC in pyroptosis *in vitro*[3] while it plays role in cytokines maturation as ASC-deficient macrophages exhibited defective maturation of IL-1β and IL-18[14]. In clearance of *S. typhimurium* via pyroptosis, there is no role of IL-1β and IL-18. Like *S. typhimurium*, clearance of *P. aeruginosa* also depends upon NLRC4 based detection of flagellin[20].

Induction of pyroptosis during *salmonellosis*

Pyroptosis is defined as caspase-1 dependant programmed cell death which is envisage being proinflammatory in nature and results in loss of cell membrane integrity as well as release of cytosolic contents. Although we know much about the mechanism of pyroptosis *in vitro* in case of *S. typhimurium* but still it needs to investigate in vivo[3, 14]. Cells can die through distinct biochemical pathways such as accidental cell death or programmed cell death and inflammatory or not inflammatory cell death. When NLRC4 is activated by cytosolic flagellin, it results in activation of pro-caspase-1 to mature caspase-1. The card domain of NLRC4 interacts with card domain of Pro-caspase-1 and this in turn results

in proteolytic processing and release of IL-1β and IL-18 as well as pyroptotic cell death. Pyroptotic cells undergo DNA fragmentation and nuclear condensation like apoptotic cells but secretion of inflammatory mediators like IL-1β and IL-18[3]. *S. typhimurium* can survive and replicate in macrophages. As a result of pyroptosis when macrophage expels bacteria, they are taken up by neutrophils and killed by reactive oxygen species. Loss of mitochondrial integrity and release of cytochrome c, which can activate apoptotic caspases, do not occur during pyroptosis[21].

Conclusion

S. typhimurium is a versatile pathogenic microorganism which uses its different effector proteins to modulate host cell signaling pathways.[8] *S. typhimurium* does not express SPI1 T3SS during systemic phase of infection, so it evades pyroptosis, an innate immune effector mechanism that would otherwise provides complete protection to the host. Now pyroptosis is viewed as physiologically important form of cell death, which expels intracellular pathogens from macrophages. Further characterization for role of pyroptosis *in vivo* will be beneficial to understand the innate immune response to different microorganisms.

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