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Salmonella-induced inflammasome activation in humans

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ABSTRACT

Inflammasomes are macromolecular complexes that assemble upon recognition of pathogen- or danger-associated molecular patterns. Inflammasome assembly is nucleated by the oligomerisation of specific, activated pattern recognition receptors within the cytosol. Inflammasomes function as platforms for the activation of the caspase-1 protease, which in turn triggers the maturation and secretion of the pro-inflammatory cytokines IL-1β and IL-18, and initiates pyroptosis, a highly inflammatory form of lytic cell death. Recently, additional inflammatory caspases (murine caspase-11, and human caspase-4/5) were also reported to be activated upon a pyroptosis-inducing 'non-canonical inflammasome' by direct recognition of lipopolysaccharide (LPS), a pathogen-associated molecular pattern. Here we review and discuss recent advances in our understanding of inflammasome-mediated host defence against *Salmonella* particularly in human cells, and their implications for cellular survival and cytokine secretion.

1. Introduction

Salmonella enterica is a flagellated enteric Gram-negative bacterium causing over 200 million cases of disease globally each year. *S. enterica* is typically ingested orally, and can be carried asymptomatically or cause enteric fever (typhoid fever), diarrhea or sepsis (Coburn et al., 2007). *S. enterica* pathogenesis depends upon both the host immune status (Gordon, 2008) and the virulence of the serovar (Fierer and Guiney, 2001). *S. enterica* serovar *typhi* (*S.* Typhi) is a human-restricted pathogen that causes typhoid fever. Typhoid fever is often modelled in mice by systemic challenge with *S. enterica* serovar Typhimurium (*S.* Typhimurium). In humans, *S.* Typhimurium generally causes self-limiting gastroenteritis but can lead to sepsis and mortality in immunocompromised individuals (Coburn et al., 2007; de Jong et al., 2012). The pathogenicity of *S. enterica* serovars is determined by various virulence factors, which are mainly encoded by *Salmonella* pathogenicity islands (SPIs) (de Jong et al., 2012). *S.* Typhimurium encodes 12 SPIs, SPI1-6, 9, 11, 12, 13, 14 and 16, but virulence largely depends on two type 3 secretion systems (T3SS) encoded by SPI1 and SPI2, which allow the injection of bacterial effectors into the host cell cytoplasm (de Jong et al., 2012; LaRock et al., 2015). SPI1 and SPI2 expression are tightly regulated and respond to environmental cues (Hansen-Wester and Hensel, 2001).

Upon human infection, *Salmonella* is recognised by the innate immune system, which provides important host defence against invading pathogens. Cells of the innate immune system such as macrophages, neutrophils and dendritic cells, express germline-encoded pattern-recognition receptors (PRRs) including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and AIM2-like receptors (ALRs). These PRRs detect extracellular and intracellular pathogens by sensing a variety of evolutionary conserved pathogen-associated molecular patterns (PAMPs) including proteins, nucleic acids, lipids and carbohydrates (Kumar et al., 2011). Signalling by most PRRs triggers pro-inflammatory pathways and antimicrobial responses, resulting in the production of a variety of cytokines and chemokines, and the upregulation of cell adhesion molecules and immunoreceptors (Akira et al., 2006). PAMP recognition by specific PRRs drives pro-inflammatory responses through the assembly of a molecular complex termed the inflammasome. The function of inflammasomes is to provide a signalling platform that activates specific inflammatory caspases (caspase-1/-4/-5 in human; caspase-1/-11 in mouse). Caspase-1, the most fully characterised inflammatory caspase, cleaves the inactive cytokine precursors pro-interleukin (IL)-1β and pro-IL-18, to yield their active secreted forms (Martinon et al., 2002; Schroder and Tschopp,

Abbreviations: AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; DAMP, danger-associated molecular pattern; GBP, guanylate-binding proteins; IL, interleukin; LPS, lipopolysaccharide; NLR, NOD-like receptor; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; SCV, *Salmonella*-containing vacuole; SPI, *Salmonella* pathogenicity island; T3SS, Type 3 secretion system; TLR, Toll-like receptor.

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2010; Monteleone et al., 2015). Activated inflammatory caspases also cleave pro-Gasdermin-D to drive pyroptosis, a lytic form of cell death that triggers inflammation (Boucher et al., 2014; Shi et al., 2015; Kayagaki et al., 2015; He et al., 2015). In this review we will highlight recent advances in the inflammasome field with a specific focus on *Salmonella* and human inflammasome biology.

2. *Salmonella* invasion of the gut epithelium

Ingestion of *Salmonella* delivers the bacterium in the gastrointestinal (GI) tract. The human gut is colonised by its natural microbiota which suppress pathogen growth and compete for nutrients (Kamada et al., 2013). In mice, paneth cells respond to invasive *Salmonella* by secreting antimicrobial peptides such as C-type lectins RegIIIβ/γ (Godinez et al., 2009; Bevins and Salzman, 2011). Secreted RegIIIγ kills other intestinal pathogenic bacteria (Brandl et al., 2008, 2007), but not *Salmonella* spp., while RegIIIβ kills various commensal bacteria (Stelter et al., 2011). Thus, it appears that *Salmonella* induces the secretion of these C-type lectins to reduce competition for nutrients and confer a growth advantage for *Salmonella* over the natural microbiota, resulting in *Salmonella* colonisation of the gut. The human orthologue of RegIIIγ, RegIIIα (also known as HIP/PAP), is bactericidal to Gram-positive bacteria but not to Gram-negative bacteria (Mukherjee et al., 2014; Cash et al., 2006), suggesting *Salmonella* may employ a similar mechanism during infection of humans.

In order to escape the hostile environment of the gut, *Salmonella* has to breach the thick mucus layer present in the GI tract to reach the underlying epithelial cells (Hansson, 2012). *Salmonella* flagella propel the bacterium against the mucus flow towards the epithelial cells (Stecher et al., 2004). To penetrate the underlying epithelial barrier *Salmonella* attaches to the epithelial cells via fimbrial adhesins and promotes bacterial uptake via epithelial membrane ruffling (Baumler et al., 1996; Takeuchi, 1967). However, *Salmonella* preferentially enters microfold (M) cells (Jones et al., 1994). These specialised intestinal epithelial cells sample the gut lumen and transcytose antigens and microorganisms to the underlying Peyer's patches (Mabbott et al., 2013).

3. Toll-like receptor detection of *Salmonella* Typhimurium

Breaching the epithelium allows *Salmonella* to evade the hostile environment of the gut, but its translocation to the underlying lamina propria allows for recognition by innate immune cells associated with the gut-associated lymphoid tissue (GALT). The GALT consists of a broad variety of leukocytes including monocytes, macrophages, dendritic cells and neutrophils (Rydstrom and Wick, 2007; Broz et al., 2012a), and these cells express a wide variety of PRRs for pathogen detection. *Salmonella* is initially sensed by TLRs, which are expressed on the cell surface and in the endosomal system of GALT and epithelial cells (Abreu, 2010).

TLRs recognise various PAMPs associated with Gram-negative bacteria, such as *Salmonella*. Such TLR-activating PAMPs include lipoprotein (TLR1/2 or TLR2/6 heterodimers) (Aliprantis et al., 1999; Takeda et al., 2002), lipopolysaccharide (TLR4) (Poltorak et al., 1998), flagellin (TLR5) (Hayashi et al., 2001) and unmethylated CpG DNA (TLR9) (Hemmi et al., 2000) (Fig. 1). PAMP-induced homo and heterodimerisation of TLRs facilitates downstream signalling via four major signalling adaptors, all of which contain a Toll/interleukin-1 (TIR) domain. These adaptors are myeloid differentiation primary-response protein 88 (MYD88), MYD88-adaptor-like protein (MAL), TIR domain-containing adaptor protein inducing IFN-β (TRIF) and the TRIF-related adaptor molecule (TRAM). TLR5 and TLR9, when stimulated with synthetic ligands, signal solely via MYD88 while TLR1/2, TLR4, TLR2/6 and TLR9 signalling induced by natural ligands additionally requires MAL for MyD88-dependent signalling (Gay et al., 2014; Bonham et al., 2014). TLR4 is unusual in that it can localise both to the cell surface and the endosome. TLR4 recognises extracellular LPS to trigger MyD88-dependent signalling at the plasma membrane, after which it relocates to the endosome to signal via TRIF and TRAM (Tanimura et al., 2008). TLR signalling generally leads to the recruitment and activation of the IL-1R-associated kinases (IRAKs) and TNF receptor-associated factors (TRAFs), and leads to the activation of transcription factors, including nuclear factor-κB (NF-κB) and members of the interferon-regulatory factor (IRF) family (O'Neill et al., 2013).

Activated NF-κB induces the transcription of pro-inflammatory cytokines such as pro-IL-1β (Lawrence, 2009). As IL-1β signalling stimulates the expression of genes associated with inflammation and autoimmune disease, the activity of this cytokine is tightly regulated to maintain homeostasis (Dinarello, 2002), and is regulated by at least two distinct signals. IL-1β is initially expressed as an inactive precursor protein, pro-IL-1β, that requires maturation for activity upon the IL-1 receptor. Pro-IL-1β is poorly expressed by most resting cells, so a priming signal (signal 1) is required for its transcriptional induction and expression. Intracellular pro-IL-1β expression is induced by TLRs or specific pro-inflammatory cytokines that activate NF-κB. Such signals also mildly promote the expression of the related cytokine, pro-IL-18 (Schroder et al., 2012a), but pro-IL-18 is constitutively expressed by many cell types (Puren et al., 1999). Pro-IL-1β and pro-IL-18 maturation to their active cytokines is controlled by the protease caspase-1 (signal 2). Mature IL-1β and IL-18 exit the cell through a poorly characterised unconventional secretion pathway (Monteleone et al., 2015).

4. Conventional inflammasomes activated by *Salmonella* Typhimurium

Active caspase-1 is generated by pro-caspase-1 recruitment to large signalling complexes, the inflammasomes. Inflammasome assembly is usually triggered by cytosolic NOD-like receptors (NLRs e.g. NLRP1, NLRP3, NLRC4, NLRP6) or inflammasome-nucleating proteins from other PRR families (e.g. AIM2, PYRIN). Inflammasome-nucleating PRR are primarily expressed by immune cells, but can also be expressed by non-immune cells such as epithelial cells (Knodler et al., 2014; Sellin et al., 2014). These PRRs surveil the intracellular environment for PAMPs and danger-associated molecular patterns (DAMP), and upon sensing such signals, nucleate the assembly of an inflammasome complex. Most inflammasomes are composed of a PRR, the apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and caspase-1 (Schroder and Tschopp, 2010). For example, danger detection by NLRP3 triggers ATP-dependent NLRP3 oligomerisation (Duncan et al., 2007) and recruitment of the ASC inflammasome adaptor protein. ASC has unusual prion-like properties; its recruitment to inflammasomes triggers its polymerisation to generate a large signalling platform known as the 'speck'. The ASC speck recruits pro-caspase-1 through homotypic interactions between ASC and pro-caspase-1 CARD domains, leading to cluster-induced dimerisation, auto-proteolysis, and caspase-1 activation (Martinon et al., 2002; Srinivasula et al., 2002). Active caspase-1 then cleaves the pro-inflammatory cytokines pro-IL-1β and pro-IL-18 into their active, secreted forms (Schroder and Tschopp, 2010). Caspase-1 is additionally required for the processing and secretion of the human anti-inflammatory cytokine IL-37 (Bulau et al., 2014) and also cleaves non-cytokine substrates. A new caspase-1 substrate, pro-Gasdermin-D, was recently identified (Agard et al., 2010) and mature Gasdermin-D was recently shown to form plasma mem-

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