The Detrimental Role of Type I Interferon Signaling During Infection with *Salmonella typhimurium*

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TLR-Dependent Induction of Type I IFNs

During infectious disease a host can recognize pathogens by various receptors unified under the term pathogen recognition receptors (PRR). These receptors initiate signaling cascades to alert the host immune system of the imminent danger associated with the invading pathogen, which commits the immune system to first restraining and ultimately, clearing off the pathogen [1]. Such signaling results in activation of the innate immune response, which in turn leads to amplification of the adaptive branch of the immune system [2]. Among the best described PRRs is the family of the Toll-like receptors (TLRs). These receptors are expressed by most if not all host cells, are localized on the host cell surface and at the endosomal compartment or both cell surface and endosomes, depending on the cell type. For recognition and activation of TLR4 by LPS, a set of adaptor proteins, MD2 and CD14, are necessary. These adaptor proteins are located extracellularly. The MD2–TLR4 complex is able to distinguish smooth or rough forms of LPS [3], where CD14 relays the signal accordingly [4]. For the rough form, the signaling through TLR4 is MyD88-dependent, and when smooth LPS serves as ligand, the TRIF-mediated signaling cascade is the dominant form of downstream gene activation [4]. The distinction between smooth and rough form is based on the oligosaccharide component of the LPS [5, 6]. LPS on the surface of *Salmonella* is of the smooth form, which suggests that the TRIF pathway is the predominant mechanism of type I IFN expression. The lipid A component of LPS is also highly inflammatory, which activates the MyD88 pathway, and synthetic structures with modification of lipid A have been shown to selectively induce the TRIF pathway [7]. Furthermore, the modifications of lipid A also determine how potent if any the signaling cascades are activated. Namely, LPS comprised of hexa- and hepta-acetylated lipid A is strongly

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inflammatory, in contrast to tetra-acetylated lipid A [8, 9]. The pathogenic *Salmonella* LPS has the smooth oligosaccharide component and the hexa-acetylated lipid A. This assures that the host cells are strongly engaged and potent inflammatory response is mounted. Type I interferon production in response to TLR4 engagement occurs predominantly through a MyD88 independent, TRIF-dependent mechanism [10].

Recent studies have shown that *Salmonella* exploits this induction of a strong inflammatory response to promote its intracellular survival [11, 12]. CpG treatment of mice that normally resolve *S. typhimurium* infection resulted in host susceptibility [12]. This was due to the enhanced intracellular proliferation of *Salmonella*, which requires expression of the *Salmonella* pathogenicity island 2 (SPI-2) genes [12]. In another study, when TLR2-TLR4-TLR9 triple knock-out mice were infected with *Salmonella*, they survived better than combinations of double knock-outs of the same TLR members [11]. Again this was shown to operate through induction of SPI-2 genes, which were induced in response to TLR engagement. Activated TLR9 recruits MyD88, IRAK1, IRAK4, and TRAF6 to phosphorylate/activate IRF7, followed by IRF7 translocation in the nucleus where it can activate type I IFNs production [13]. This is summarized in Fig. 1.

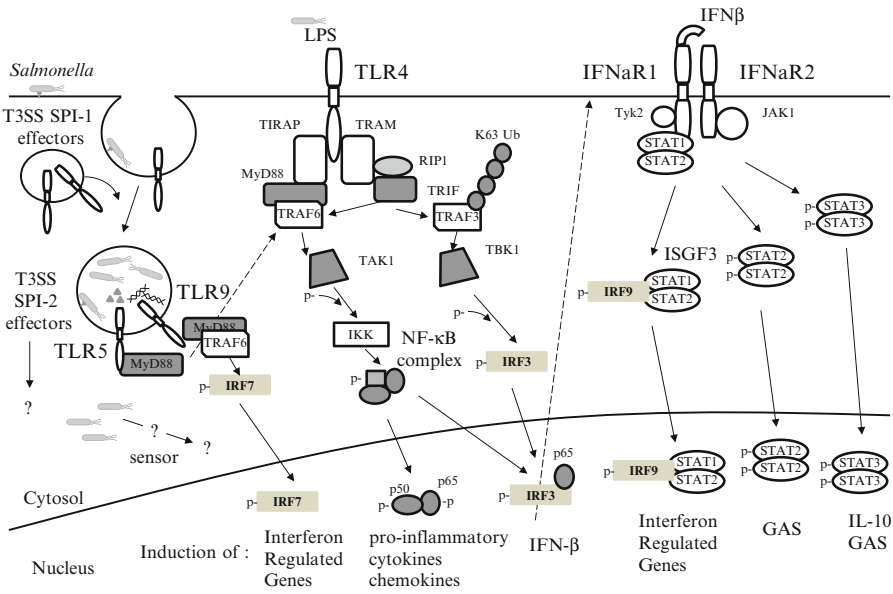

Fig. 1 Major induction pathways of type I IFNs by *Salmonella* and role of type I IFN during infection at the cellular level. *Salmonella* SPI-1 effectors induce its engulfment in a SCV or phagosome where the SPI-2 effectors get induced. Although many functions are described for SPI-2 effectors, it remains unclear whether they regulate type I IFN production. Once in the phagosome or SCV, activated TLR9 can relay signals to IRF7 to stimulate IRGs and the TLR5 similar to TLR4 via MyD88 pathway activates the NF-κB complex. LPS activated TLR4, signals through MyD88 or TRIF-dependent pathways. The MyD88 pathway, via activated NF-κB, leads to induction of proinflammatory cytokines and chemokines and the TRIF pathway leads to IRF3 activation and type I IFN production. The type I IFN produced then engages the IFNAR to induce production of several hundreds of interferon regulated genes or gamma-activated sequences (GAS) via autocrine loop. *IKK* inhibitor of NF kappa-B kinase, *TRAF* tumor necrosis factor receptor-associated factor

Type III Secretion System-Dependent Induction of Type I IFN

Salmonella infects various types of cells. While phagocytic cells such as macrophages and dendritic cells can rapidly phagocytose *Salmonella*, the non-phagocytic cells are infected through a type III secretion system (T3SS) encoded in the SPI-1 cluster of genes. The T3SSs are needle-like structures canonically used by bacteria to bridge bacterial cytoplasm with the host cytosol and translocate proteinaceous effector molecules, which in case of pathogenic bacteria subvert host cell signaling [14]. The SPI-1 induces host cell structures that promote engulfment of *Salmonella* and its intracellular translocation into vacuoles, termed *Salmonella* containing vacuoles, SCVs. Professional phagocytic cells don't require SPI-1 to phagocytose *Salmonella*, and once intracellular, the host could potentially recognize other pathogen-associated molecular patterns (PAMPs) beside LPS. However, SPI-1 is active in the phagocytic cells as well. PrgJ, a capping protein of the T3SS of SPI-1, gets removed from the needle structure of T3SS and enables secretion of *Salmonella* effectors. This allows *Salmonella* to engage the NLRC4 inflammasome [15]. Flagellin, which is expressed by *Salmonella*, and is needed for its virulence, serves as a signal for TLR5 and NLRC4 inflammasome engagement, which in turn leads to activation and production of proinflammatory cytokines [16]. The flagellum is evolutionary related to the T3SS machinery and in certain conditions can secrete proteins as well [17, 18]. By engaging the inflammasomes, the production of active IL-1β is maintained, which is able to positively feed into the type I IFN production by inhibiting the DUBA, deubiqutinase known to remove K63 ubiquitination of TRAF3 [19]. K63 ubiquitination of TRAF3 is a major modification required for IFN gene expression [20].

In a study that addressed the role of caspase-8 during *Salmonella* infection it was shown that caspase-8 is recruited to the inflammasome complex. This recruitment was shown to be specific to *S. typhimurium* infection and as part of that complex it contributed positively to IL-1β production [21]. The production of active IL-1β seems to be fine-tuned, as it is shown that SipB, a *Salmonella* SPI-1 effector protein, promotes its production [22]. Active IL-1β has many other functions, yet the IL-1R signaling by modulating TRAFs remains instrumental for type I IFN production [13, 20]. It is important to note that IL-1 signaling can also accelerate the degradation of IFNAR by activating kinases that add phospho-moiety to a so-called degron sequence within the IFNAR protein [23], therefore adding complexity to the role of IL-1 signaling in type I IFN production and signaling.

Microarray studies focused on the host response to *Salmonella* infection revealed that many genes are specifically activated. RAW24.7, a murine macrophage cell line infected by *S. typhimurium*, was assessed for gene expression. The following genes were found to be upregulated: MIP-1α, MIP-1β, MIP-2α, IL-1β, TNF receptor, CD40, IκBα, IκBβ, NF-E2, IRF1, and c-rel among many [24]. In a similar study it was shown that SPI-1 effectors exploit host pathways that are independent of TLR engagement. Many genes in uninfected control remained at same expression level as cells infected by SPI-1 mutant *Salmonella* strain [25]. In that same study STAT3,

a transcriptional factor with pleiotropic effects was upregulated [25], which in cooperation with IRF1 regulated the production of IL-10 [26]. Indeed, IL-10 is an anti-inflammatory cytokine, which has been shown to promote the intracellular proliferation of *Salmonella* [27].

Role of Type I IFNs During *Salmonella* Infection

In various infectious disease models (e.g., *Listeria*, *Mycobacteria*, *Trypanosoma*, *Candida*), it has been shown that IFNAR-deficient mice display enhanced survival [28–31]. Similarly, IFNAR-deficient mice display enhanced survival during infection with virulent *S. typhimurium* [32]. It is conceivable that pathogens from different domains of life or classes have converged in utilizing mechanisms of subverting the host immune defenses, and the above-mentioned examples would reiterate the importance of type I IFN signaling in host–pathogen interactions. The complexity of interferon signaling pathways and its impact on *Salmonella* pathogenesis was further revealed in another study in which UBP43-deficient mice (alternatively known as USP18) were shown to have elevated type I interferon signaling, yet these mice were able to control *Salmonella* better in vivo, since the splenic bacterial burden was reduced in UBP43-deficient mice; however, there was no difference in host susceptibility between WT and UBP43-deficient mice [33, 34]. UBP43 is a member of the "Ubiquitin specific protease" family that cleaves ISG15, a ubiquitin-like posttranslational modification (PTM) of proteins, which appears to be dependent on IFN-signaling [35]. The mechanism behind the better control of *Salmonella* in UBP43-deficient mice was attributed to the sustained and hyperactive JAK-STAT1 signaling, as the failure to remove ISG15 from the JAK1 resulted in prolonged JAK1-STAT1 signaling [36]. Furthermore, UBP43-deficient mice displayed elevated expression of genes that are dependent on type I IFN signaling (ISGs), and were hypersensitive to LPS-induced septic shock [33]. While these results may appear to be at odds with the phenotype obtained in IFNAR-deficient mice, however, the UBP43 deficient mice display elevated inflammatory signaling in contrast to IFNAR-deficient mice. Elevated inflammatory signaling in UBP43-deficient mice may promote initial clearance of bacteria, but the overt inflammatory response may lead to fatality at a later time period. Work on *Salmonella* invasiveness after treatment with type I IFN, suggests that epithelial cells are less susceptible to invasion [37], and because of that impaired invasion it is argued that mice challenged intragastrically with *Salmonella* show enhanced survival if treated with type I IFNs [38].

Furthermore type I IFN signaling is implicated in the regulation of inflammasome activation, and stimulation of necrosome formation, both presently understood as distinct signaling complexes. Inflammasomes are protein complexes that enable activation of inflammatory caspases, which drive immune responses by stimulating the production of proinflammatory cytokines, and by inducing pyroptosis, a mechanism of proinflammatory cell death [39]. Work done on elucidating the mechanisms

involved in inflammasome regulation by IFNAR signaling indicated that type I IFN inhibits the production of IL-1β, through regulation of the NLRP3, leading to reduced transcript levels of pro-IL-1β [40]. Yet still, during infection with gram-negative bacteria, type I IFN promotes IL-1β production by controlling caspase-11 activity [41], and most likely such duality is dependent on the amount of IFN-β.

Necrosome is a protein complex that when assembled leads the host cell to necroptosis, a proinflammatory mechanism of cell death. Typically it is induced by TNFα-TNFR1 interaction in the absence of apoptosis [42]. During *S. typhimurium* infection of macrophages it was shown that type I IFN signaling stimulates necrosome activation leading to necrotic cell death, where IFNAR KO bone marrow macrophages showed enhanced survival [32]. Type I IFN signaling is the critical check-point of necrosome activation in macrophages. During in vivo infection, IFNAR-deficient mice had more macrophages, which correlated to better control of *Salmonella*. Additionally, the abrogated cytokine signaling downstream of IFNAR can also be a contributing factor, as the pleiotropic effects of IFN signaling can modulate subsequent downstream cytokine and chemokine signaling. Necroptosis is induced by IFN-α/β and IFN-γ signaling pathways independent of death receptors signaling, but dependent on Protein kinase RNA-activated (PKR) and Fas-associated death domain (FADD) [43]. Further, even TNF-dependent necrosome activation appears to be dependent on type I IFN signaling (S. Sad, unpublished).

A hallmark of necroptosis is the release of damage associated molecular patterns (DAMPs) that can act as "secondary" ligands during host–pathogen interactions and can become major drivers of inflammatory responses, although their contribution is often neglected. Necrosome activation that is associated with *Salmonella* infection that is notorious for inducing host cell death generates overt pathology leading to adverse outcome. During infections by pathogens that are able to inhibit caspases, necroptosis can be regarded as a backup mechanism that initiates inflammatory cell death and alerts the immune system defenses. Specifically, in *Salmonella* infection, the outcome and progression are multifactorial and will not be only dependent on type I IFN signaling [44], yet the IFNAR-deficient mice show significantly reduced susceptibility to *Salmonella* infection [32].

Final Remarks

New pathways of type I IFN signaling have emerged that seem to indicate that the impact of type I IFN signaling may be highly dependent on the disease context [45]. The IFNAR KO mice have been used extensively in many studies and have revealed both the positive and negative role of type I IFN signaling. At the cellular level the role of type I IFN signaling is also complex. Resistance to LPS shock is mediated by ablation of type I IFN signaling, as IFNAR1 KO, but not the IFNAR2 KO, mice are resistant to LPS [45, 46]. Type I IFN appears to be a key mechanism that impacts inflammasome and necrosome activation, although the precise mechanistic details are lacking currently. These two distinct signaling complexes, inflammasome and

necrosome, might have substantial cross-talk since both are controlled by type I IFN. *Salmonella* is a chronic intracellular pathogen, which results in persistent activation of immune response. It is therefore quite conceivable that type I IFN signaling plays a key role in this process, which results in a deleterious host outcome due to persistent pathology.

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