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Mini review

Inhibition of death receptor signaling by bacterial gut pathogens

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ABSTRACT

Gastrointestinal bacterial pathogens such as enteropathogenic *Escherichia coli*, *Salmonella* and *Shigella* control inflammatory and apoptotic signaling in human intestinal cells to establish infection, replicate and disseminate to other hosts. These pathogens manipulate host cell signaling through the translocation of virulence effector proteins directly into the host cell cytoplasm, which then target various signaling pathways. Death receptors such as TNFR1, FAS and TRAIL-R induce signaling cascades that are crucial to the clearance of pathogens, and as such are major targets for inhibition by pathogens. This review focuses on what is known about how bacterial gut pathogens inhibit death receptor signaling to suppress inflammation and prevent apoptosis.

1. Introduction

Bacterial pathogens activate a number of signaling cascades within host cells during infection, many of which subsequently induce inflammation. Alternatively, and often in parallel, microbial detection can activate apoptotic signaling, which leads to the eradication of infected cells. The benefits of inhibiting or inducing cell death or inflammation for a pathogen differ depending on the specific pathogen and type of host cell targeted. For some pathogens, the induction of cell death in epithelial cells facilitates invasion to deeper tissues, while inducing cell death in immune cells can promote pathogen survival [1]. Bacterial gut pathogens have evolved highly specific mechanisms to modulate cell death and inflammatory signaling pathways in order to successfully establish infection, replicate and disseminate to other hosts. Ultimately, the inhibition of inflammation allows the pathogen to evade the innate immune response. However, inflammation can also be useful to pathogens, for example *Salmonella* induces inflammation to outcompete commensal bacteria in the gut [2].

Inflammation and cell death are induced by a variety of extrinsic and intrinsic factors targeting different cellular receptors. Key signaling pathways involved in the host anti-microbial defences include the nuclear factor-kappa B (NF-κB) transcriptional regulator and mitogen-activated protein kinase (MAPK) pathways. This review will focus on how bacterial gut pathogens inhibit death receptor signaling to prevent inflammation and host cell death.

2. Death receptors and bacterial gut pathogens

Death receptor signaling is a significant component of the host response to bacterial gut pathogens. Death receptors including TNFR1, FAS (TNFRSF6) and the TRAIL (TNF-associated apoptosis-inducing ligand) receptors, DR4 and DR5, are defined by the presence of a cytoplasmic death domain (DD), which recruits DD-containing adapter proteins to an oligomeric signalosome *via* homo- and heterotypic DD interactions [3,4]. The stimulation of death receptors occurs through extracellular cysteine-rich domains (CRD) leading either to an inflammatory response or death of the cell.

In response to TNF, TNFR1 recruits adapter proteins to form different signaling complexes that have distinct and diverse outcomes [3]. Complex I requires binding of TRADD to TNFR1 *via* DD interactions, followed by recruitment of TRAF2, RIPK1 and cIAPs to the receptor complex. This signaling platform results in the activation of NF-κB and MAPK signaling, inducing an inflammatory response [5]. Complex IIa is formed upon dissociation of TRADD from TNFR1, recruitment of FADD and procaspase-8 to TRADD, followed by the activation of caspase-8 and apoptosis of the cell. Complex IIb leading to necroptosis is formed upon de-ubiquitination and phosphorylation of RIPK1 and involves the components, RIPK3, FADD and procaspase-8 [6]. The formation of each of these signaling complexes is tightly regulated so that not all complexes can be activated at once and tissue homeostasis is maintained.

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In the canonical extrinsic apoptosis pathway, recognition of FAS ligand (FasL) by FAS leads to the recruitment of FADD and procaspase-8 and formation of the death-inducing signaling complex (DISC), which initiates cell death though the activation of caspase-8 [7]. The signaling pathway in lymphoid cells differs slightly to that of non-lymphoid cells. For the latter, processing of the pro-apoptotic protein Bid is required to induce cell death [8,9].

TRAIL-R is another death receptor which upon binding of the ligand TRAIL, recruits FADD and procaspase-8 to form the DISC [10]. TRAIL has been studied extensively in the context of tumor cell apoptosis, but a role for TRAIL during infection with bacterial pathogens is not well established. While the involvement of TRAIL-R and FAS in apoptotic signaling is well accepted, their potential influence on anti-apoptotic, inflammatory and pro-survival signaling are controversial. Non-apoptotic signaling *via* these receptors seems to involve NF-κB and MAPK pathways, but the physiological relevance remains unclear [10,11].

The evolution of bacterial pathogens to inhibit death receptor signaling can be attributed to the acquisition of virulence genes on mobile genetic elements such as prophages and integrative elements, which can be horizontally transferred between bacteria. Enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) are extracellular pathogens that infect epithelial cells of the human gut. EPEC and EHEC utilize a type III secretion system (T3SS) to inject virulence effector proteins directly into host cells, which manipulate host cell function [12]. One such effector, termed the translocated intimin receptor (Tir) mediates the formation of attaching and effacing (A/E) lesions which are characterized by intimate attachment of the bacteria to host cells and the effacement of brush-border microvilli around the adherent bacteria. The T3SS and several effectors, including Tir, are encoded by the locus of enterocyte effacement (LEE) pathogenicity island. There are also non-LEE encoded (Nle) effector proteins, many of which inhibit inflammation and cell death by blocking death receptor signaling. The effects of these proteins during infection *in vivo* have been studied using *Citrobacter rodentium*, an A/E pathogen of mice that is highly related to EPEC and EHEC.

Unlike EPEC, *Salmonella enterica* serovar Typhimurium is an invasive gastrointestinal pathogen that possesses two T3SSs encoded by *Salmonella* pathogenicity islands 1 and 2 (SPI-1 and SPI-2). While the SPI-1 T3SS is required to facilitate entry into host cells, the SPI-2 T3SS is required to establish intracellular replication. However, similar to EPEC, S. Typhimurium has evolved to evade host immune defenses through the injection of T3SS effectors that subvert innate immune and apoptotic signaling pathways.

Shigella is the causative agent of bacillary dysentery, or shigellosis, an invasive infection of the human colon. While highly genetically related to *E. coli*, *Shigella* spp. are different to A/E pathogens as they are invasive gastrointestinal pathogens. Once inside the cell, the bacteria lyse the endocytic vacuole, replicate in the cytoplasm and spread to adjacent cells *via* the polymerization of F-actin at one pole. *Shigella* also uses a T3SS to translocate effector proteins directly in the host cell cytosol that are essential for invasion, vacuolar escape, and cell-to-cell spread [13]. As with A/E pathogens and *Salmonella*, a number of additional T3SS effectors target inflammation and cytoskeletal dynamics to promote the survival and dissemination of the pathogen [13,14]. Many of these effectors share significant sequence homology with T3SS effectors of EPEC, EHEC and *Salmonella* and are likely to have similar functions within the cell.

3. Inhibition of death receptor induced inflammation

Gut bacterial pathogens trigger innate immune signaling *via* recognition of their pathogen associated molecular patterns (PAMPs) including flagellin and LPS [15]. Inflammatory cytokines such as TNF can then induce death receptor signaling and further inflammation *via* the activation of NF-κB or MAPK pathways [5].

Early studies showed that while EPEC PAMPs induce inflammation, the pathogen possesses the ability to inhibit the production of inflammatory cytokines [16,17]. Prior EPEC infection led to the inhibition of IL-8 production in infected cells even when stimulated with TNF, IL-1β or bacterial flagellin. The inhibition was T3SS dependent, and subsequently several effectors of EPEC and EHEC were shown to inhibit NF-κB signaling by targeting different host cell components using diverse mechanisms of action (Table 1).

TNF produced during *Shigella* and *Salmonella* infection also triggers MAPK and NF-κB activation. Indeed, patients infected with *S. dysenteriae* or *S. flexneri* have consistently higher levels of cytokines including TNF in their serum, intestinal tissue and stools during both the acute and convalescent phase of infection [18,19]. Likewise, increased levels of inflammatory cytokines such as TNF are observed in sera from patients suffering from gastrointestinal *Salmonella* infections [20]. Similar to EPEC, T3SS effectors from *Shigella* and *Salmonella* have been described that inhibit inflammatory signaling pathways (Table 1).

3.1. Inhibition of NF-κB signaling by bacterial T3SS effectors

3.1.1. Targeting of TAB2 and TAB3

NleE is a T3SS effector of A/E pathogens that blocks NF-κB signaling in response to TNF and IL-1β. Initial studies showed that cells infected with A/E pathogens or expressing NleE ectopically were unable to respond to stimulation with TNF or IL-1β and that NleE prevented IκB degradation and p65 nuclear translocation [21,22]. Recently, NleE was shown to target the adapter proteins TAB2 and TAB3 upstream of IκB in the NF-κB signaling pathway [23] (Fig. 1). NleE is a novel cysteine methyltransferase that modifies TAB2 and TAB3 by transferring a methyl group onto a zinc coordinating cysteine residue within the Npl4 zinc finger domain. This prevents recognition of the ubiquitin chains on TRAF2 and TRAF6, the ubiquitin ligases involved in the TNFR1 and IL-1 receptor complexes respectively [23]. The activity of NleE depends on a conserved six amino acid motif, ^{209}IDSYMK^{214}, within the C-terminal region that is essential for the effector to block NF-κB activation and modify TAB2/3 [22,23]. Although several EPEC effectors inhibit NF-κB signaling, NleE appears to contribute significantly to the prevention of IL-8 secretion during infection of epithelial cells [22]. However, despite the potency of its activity *in vitro*, the importance of NleE during infection *in vivo* has been hard to define. During *C. rodentium* infection of mice, *nleE* null mutants show only a marginal defect in virulence in comparison to wild-type *C. rodentium* infection [24,25], perhaps due to redundancy in activity with other T3SS effectors.

OspZ is a homologue of NleE, found in all *Shigella* species that also inhibits NF-κB activation and p65 nuclear translocation [22] (Fig. 2). Given the high amino acid sequence similarity with NleE in all species except *S. flexneri* serotype 2a [22], OspZ presumably also exhibits methyltransferase activity and targets TAB2/3 during *Shigella* infection. Curiously, OspZ from *S. flexneri* 2a is truncated by 36 amino acids at the C-terminus, lacks the IDSYMK motif and is non-functional [22,26]. The non-functional form of OspZ is highly conserved among strains of *S. flexneri* 2a and it is unclear why the truncated gene is maintained in the bacterial genome.

3.1.2. Control of cellular ubiquitination by type III effectors

Ubiquitination is a key mechanism regulating many eukaryotic cellular processes, including cell cycle progression, gene transcription and death receptor signaling [27]. The *Shigella* effector OspG, is an atypical Ser/Thr protein kinase that inhibits NF-κB activation by preventing ubiquitination and subsequent proteasomal