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Pathogenic *Salmonella* species are enteroinvasive gram-negative bacteria that produce a number of clinical manifestations in humans and animals. The bacteria enter the body through the oral route, penetrate the intestinal epithelium and depending upon the *Salmonella* serovar and/or the type of the host, either cause localized gastroenteritis or disseminate throughout the reticuloendothelial system. The invasion of intestinal epithelial cells (IECs) is brought about by a specialized protein secretion apparatus called the type III secretion system expressed by *Salmonella* and many other pathogenic bacteria (Kubori et al., 2000). The interaction of *Salmonella* with IECs induces secretion of inflammatory cytokines and chemokines which bring about recruitment of inflammatory cells including neutrophils, mononuclear phagocytes and dendritic cells to the site of infection. One or more of these cell types is believed to cargo *Salmonella* from the gut to secondary lymphoid organs (Cotter and DiRita, 2000). A large number of inflammatory responses produced during interaction of *Salmonella* with IECs, dendritic cells and macrophages are generated through activation of Toll-like receptors (TLRs) on these cells. These responses are also vital for host defense against this pathogen.

TLRs recognize a large number of structurally unrelated molecular patterns present amongst microorganisms. These patterns include molecules like LPS and peptidoglycan that are important components of bacterial cell wall, flagellin that is required for bacterial mobility and viral RNA & bacterial DNA, indispensable molecules of the genetic machinery (Ramos et al., 2004; Takeda et al., 2003). Toll was first identified in Drosophila as a gene whose product was essential for dorso-ventral pattern development in the fly (St Johnston and Nusslein-Volhard, 1992). Subsequent studies showed that this molecule was also required for anti-fungal and anti-bacterial defence in flies (Lemaitre et al., 1996).
Studies in mammals have led to the identification of 13 such receptors. These receptors are expressed on many cell types including gut epithelial cells, macrophages, dendritic cells, lymphocytes and cells lining the respiratory and urogenital tracts (Akira et al., 2006; Zhang et al., 2004; Hornung et al., 2002). Activation of these receptors with PAMPs (pathogen associated molecular patterns) initiates inflammatory and innate immune responses that play a vital role in clearance of pathogens and in linking innate immunity with adaptive immunity (Iwasaki and Medzhitov, 2004).

LPS and flagellin play a major role in producing inflammatory and innate immune responses during infection with *Salmonella*. These two molecules are recognized by TLR4 and TLR5 respectively. Flagellin is particularly important at the level of intestine as TLR4 remains downregulated in IECs (Abreu et al., 2001). TLR5 is expressed on intestinal epithelial cells, kidney epithelial cells, lung epithelial cells, mononuclear phagocytes, dendritic cells and T lymphocytes. In IECs it is predominantly present on the basolateral side and therefore gets activated mainly by those pathogens that are able to deliver flagellin across the epithelium. Flagellin stimulates IECs to secrete a large number of chemokines including IL-8 and MIP-2α (Hybiske et al., 2004; Berin et al., 2002; Eaves-Pyles et al., 2001; Gewirtz et al., 2001; Sierro et al., 2001; Steiner et al., 2000). IECs also upregulate expression of inducible nitric oxide synthase (iNOS), matrilysin [matrix metalloproteinase] and β-defensin 2 upon incubation with flagellin (Zeng et al., 2003; Sierro et al., 2001; Steiner et al., 2000). In mice, TLR5 has been shown to be required for systemic dissemination of *S.typhimurium* and for generation of gut immune responses against the pathogen (Uematsu et al., 2006). TLR5 is also important for immunity against uropathogenic *E.coli, Legionella* and *Pseudomonas*. In addition to its ability to generate inflammatory responses from epithelial cells, flagellin can also induce secretion of
cytokines and chemokines from macrophages and dendritic cells and also bring about up-
maturation of human DCs as well as upregulation of MHC class II, CD80 and CD86
(Iwasaki and Medzhitov, 2004). More recent studies have shown that flagellin can co-
stimulate cytokine secretion from activated human T cells and modulate functions of T
regulatory cells (Crellin et al., 2005). Flagellin also activates inflammatory responses
through interaction with the inflammasome component Ipaf (Franchi et al., 2006; Miao et
al., 2006); it is currently under development as an adjuvant.

The binding of flagellin to TLR5 brings about dimerization of the receptor and
activation of intracellular signaling that is dependent on the adaptor protein MyD88. This
signaling cascade, which involves IRAK4, IRAK1 and TRAF6 (Gohda et al., 2004; Khan
et al., 2004; Picard et al., 2003; Moors et al., 2001), brings about translocation of NF-κB to
the nucleus (Berin et al., 2002; Eaves-Pyles et al., 2001) and activation of MAP-kinases
resulting in the production of inflammatory and innate immune responses. Remarkably,
activation of TLR5 takes place only upon ligation of the receptor with monomeric flagellin.
The polymeric form (the flagellum) which is the primary form in which flagellin exists on
bacteria is unable to bind TLR5 and therefore unable to activate cells. This has been shown
to be due to inaccessibility of receptor binding region in the polymeric filament. This has
raised the question as to how flagellin monomer might bring about dimerization of TLR5.
It has been suggested that a single monomer of flagellin might bind TLR5 asymmetrically
or two flagellin monomers might bind the receptor symmetrically or flagellin might
employ a co-stimulatory molecule to activate responses through TLR5. However, the
mechanism is still not understood. Moreover, while there is a lot of information available
about how inflammatory responses induced through TLR4 are regulated, there is very little
known about how TLR5 – mediated responses might be regulated. The present study
analyzed the role of co-factors in the regulation of flagellin – induced cellular responses and investigated the mechanism by which flagellin might co-stimulate cytokine secretion from activated T cells.