Introduction
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Pathogenic *Salmonella* species produce a number of clinical manifestations in humans and animals. *Salmonella typhi* causes systemic infection, typhoid, exclusively in humans. On the other hand non-typhoidal *Salmonella* species such as *S.typhimurium* have broader host specificity. *S.typhimurium* produces localized gastroenteritis in humans and a systemic disease in mice. Salmonellae enter the body through the oral route, penetrate the intestinal epithelium and depending on the *Salmonella* serovar and/or the type of the host, may either disseminate into the reticuloendothelial system or remain localized in the gut. The invasion of intestinal epithelial cells (IECs) is an essential step for the pathogenicity of *Salmonella* and involves an intimate interaction between the bacterium and the host cell which results in a cross-talk through biochemical signals. Entry of *Salmonella* into IECs (and other non-phagocytic cells) requires intact motility and secretion of invasion-promoting molecules which are components of the host cell contact-dependent type III secretion system, largely encoded on a 35-40 Kb region of the *Salmonella* chromosome located at centisome 63 (Galan and Collmer, 1999; Galan, 1999; Hueck, 1998). IECs respond to bacterial adherence / invasion by producing a number of cytokines and chemokines, which recruit a wide variety of effector cells including neutrophils, lymphocytes, monocytes and dendritic cells (DCs) to the site of infection. The magnitude and the kind of cytokines triggered from IECs depend on the pathogen encountered. Many, if not all, of the cytokines from *Salmonella*-infected IECs are produced following activation of Toll-like receptors (TLRs) by conserved pathogen associated molecular patterns (PAMPs) such as flagellin (Ramos *et al.*, 2004; Hayashi *et al.*, 2001; Eaves-Pyles *et al.*, 2001; Ciacci-Woolwine *et al.*, 1998). Flagellin-deficient *Salmonella* is unable to evoke inflammatory responses from IECs *in vitro* (Zeng *et al.*, 2003). Moreover, cellular responses produced upon infection of IECs
with *Salmonella* can be reproduced with purified flagellin (Zeng *et al*., 2003). Flagellin is therefore the major proinflammatory determinant of pathogenic *Salmonella*. It produces cellular responses by activating Toll-like receptor-5 (TLR-5) which is expressed by many cell types including IECs, macrophages, DCs and T lymphocytes (Didierlaurent *et al*., 2004; Means *et al*., 2003; McSorley *et al*., 2002; Gewirtz *et al*., 2001). TLR-5 has assumed more significance in the gut because the expression of TLR-4 which recognizes LPS, another highly conserved PAMP, is downregulated at this mucosal site (Smythies *et al*., 2005; Abreu *et al*., 2001; Smith *et al*., 2001). The responses produced from IECs in response to activation with flagellin play a major role in recruiting inflammatory cells to the site of infection (Ramos *et al*., 2004) while responses generated from DCs and macrophages constitute an important component of innate immunity against *Salmonella* (Wick, 2004). Flagellin has also been recently shown to modulate functions of T regulatory cells from humans (Crellin *et al*., 2005). All these studies point to a very important role for flagellin in the regulation of innate and adaptive immune responses during infection with *Salmonella*. Remarkably, flagellin can be recognized by its receptor TLR-5 only in the monomeric form and not when it is assembled into flagella (polymeric flagellin). This is because TLR-5 recognizes the highly conserved N and C termini of flagellin that are hidden in the flagellar filament (Smith *et al*., 2003). Since flagellin primarily appears on the bacterial surface in the form of polymeric flagella, it is not clear how monomeric flagellin becomes accessible for sensing by the innate immune system. The present study has addressed this question in detail.

Following invasion of the gut epithelium, *Salmonella* disseminates into secondary lymphoid organs such as the mesenteric lymph nodes, spleen and bone marrow, leading to systemic infection. Macrophages have long been regarded as the primary target of
Salmonella infection. Salmonella-infected mice show a rapid increase in the number of splenic macrophages (Kirby et al., 2002), and bacteria can be readily detected in these cells (Salcedo et al., 2001). Infection of macrophages with pathogenic Salmonella results in the induction of inflammatory responses through activation of TLRs, including TLR-4 (Vazquez-Torres et al., 2004; Royle et al., 2003; Heinrich et al., 2001; Zirk et al., 1999) and TLR-5 (Mizel et al., 2003; Moors et al., 2001; McDermott et al., 2000; Ciacci-Woolwine et al., 1998); it also leads to caspase-1-dependent release of the proinflammatory cytokines IL-1β and IL-18 and macrophage cell death (Maraithasan et al., 2004; Boise and Collins, 2001; Brennan and Cookson, 2000; Hersh et al., 1999; Thornberry et al., 1992). Induction of macrophage cell death is believed to be a crucial part of the innate immune response during Salmonella infection (Hueffer and Galan, 2004); however, the mechanism by which apoptosis regulates innate immunity remains unclear. This study has investigated a possible link between infection-induced cell death and regulation of innate immunity during infection of macrophages with Salmonella.

The molecules that are required for invasion of the intestinal epithelium or for the induction of inflammatory responses are very similar in different Salmonella species which suggests that these pathogens might utilize similar mechanisms to initiate these processes. However, in spite of this similarity many Salmonella species exhibit a remarkable degree of host specificity. S.typhi causes disseminated disease, typhoid fever, whereas S.typhimurium causes a relatively localized gastroenteritis in humans (House et al., 2001; Jones and Falkow, 1996). These different clinical outcomes following infection with these two closely related Salmonella species could be due to differences in the interaction of these pathogens with either IECs or with cargo cells such as DCs and macrophages that are believed to carry these bacteria to secondary lymphoid organs, or
both. *Salmonella* is known to be internalized by DCs (Hopkins *et al*., 2000) which are enriched at the site of entry of *Salmonella* in the Peyer's Patches (Wick, 2002), and are ideally placed to sample antigens. DCs, the most efficient type of antigen-presenting cells, are also known to express TLRs (Kadowaki *et al*., 2001), and form a key link between innate and adaptive immunity. *Salmonella* is believed to modulate DC functions for its survival (Qimron *et al*., 2004; Tobar *et al*., 2004; Niedergang *et al*., 2000); however the possibility that *S*. *typhi* and *S*. *typhimurium* may differently modulate the strength and quality of DC responses to influence the outcome of the infection is currently unexplored.

An important feature of DCs in the gut is that these cells are present in close association with IECs, which are believed to govern DC functions either through direct cell-to-cell contact or through soluble mediators. Mucosal DCs are conditioned by epithelial cell-released thymic stromal lymphopoietin (TSLP) and other factors to polarize T cells towards a non-inflammatory T helper type 2 (Th2) phenotype, even after exposure to *S*. *typhimurium*, a T helper 1 (Th1)-inducing pathogen (Rimoldi *et al*., 2005). In this manner, the crosstalk between IECs and DCs is believed to regulate intestinal homeostasis. This control of immune responses can, however, be disrupted during inflammation as seen in Crohn's disease (Rimoldi *et al*., 2005). Alterations in expression of epithelial cell-secreted factors and inflammatory cytokines released from IECs following encounter with pathogens may influence the functions of underlying DCs, and this modulation would depend on the pathogen encountered by the intestinal epithelium. The third part of this study has analyzed if *S*. *typhi* and *S*. *typhimurium* can differently modulate DC functions either directly, or via mediators produced following infection of IECs with these pathogens, with the aim of finding a possible explanation for different manifestations produced by these two closely related *Salmonella* species in humans.