CHAPTER 5

Intestinal epithelial cell-Dendritic cell crosstalk:

S. typhi-infected IECs promote an inflammatory DC phenotype
5.1. INTRODUCTION

*Salmonella enterica* includes several closely related serovars that cause disease in humans and animals. Some *Salmonella* serovars can infect a wide variety of hosts, while others show restricted host specificity. For example, *S. typhi* causes a systemic infection, typhoid, in humans but not in mice. In contrast, *S. typhimurium* causes localized gastroenteritis in humans, and a systemic infection in mice (House *et al.*, 2001; Jones and Falkow, 1996). Genome sequences of these two pathogens show more than 90% similarity (Edwards *et al.*, 2002; McClelland *et al.*, 2001; Parkhill *et al.*, 2001). It is believed that horizontal gene transfer has provided the genome of these bacteria with 10-12% of unique DNA, which might be responsible for the unique virulence attributes and host restriction exhibited by these two closely related pathogens. However, the specific host-pathogen interactions responsible for these different manifestations have not been identified. It is not clear whether the differences that might be responsible for different clinical outcomes produced by these two *Salmonella* species in humans, are at the level of their interaction with IECs, the first cell type that *Salmonella* invades during infection, or due to differences in the way these two pathogens interact with macrophages or DCs, cell types that might be responsible for systemic dissemination of the pathogen, or at both levels. The molecules that are important for invasion of IECs are to a large extent conserved between these two *Salmonella* serovars which suggests that the two pathogens might penetrate IECs by a similar mechanism (Edwards *et al.*, 2002; McClelland *et al.*, 2001; Parkhill *et al.*, 2001). However, one important difference that has been reported is that *S. typhi* but not *S. typhimurium* can use the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) to invade IECs (Pier *et al.*, 1998). It has also been demonstrated that processed LPS produced following interaction of *S. typhi* with host cells serves as the ligand for CFTR on
epithelial cells (Lyczak et al., 2001). However, the contribution of this specific S.typhi-CFTR interaction in typhoid pathogenesis is not clear.

The interaction of S.typhi with host cells is poorly understood primarily due to non-availability of a suitable animal model. Moreover, in vitro studies of S.typhi with immune cells from human peripheral blood have also been limited. Therefore, most of our current understanding of S.typhi-host cell interaction is based on studies carried out with S.typhimurium, which in mice is believed to cause a disease analogous to human typhoid fever. While this model has considerably increased our understanding of host-pathogen interaction during infection with salmonellae in general (Jones and Falkow, 1996), the interactions responsible for host specificity exhibited by S.typhi remain poorly understood. In addition, the inability of S.typhimurium to produce typhoid-like manifestations in humans suggests that conclusions about S.typhi-host cell interaction based on studies carried out with S.typhimurium in mice should be interpreted cautiously. S.typhimurium infection in humans is believed to produce a potent inflammatory response in the gut that brings about recruitment of inflammatory cells such as neutrophils into the intestinal lumen and consequently clearance of bacteria from the gut (Criss et al., 2001; McCormick et al., 1995a; McCormick et al., 1993); S.typhi does not elicit this neutrophil influx and its interaction with the gut is less inflammatory than S.typhimurium (McCormick et al., 1995b), which may allow for its dissemination and persistence in the human host. These differences in the magnitude and possibly the quality of inflammatory and innate immune responses produced during infection of IECs with these two closely related pathogens might determine the course of infection in humans.

The mechanism by which pathogenic Salmonella disseminates into the reticuloendothelial system is not clear. However, the process of invasion of IECs mediated
via delivery of effector molecules is believed to be an effective mechanism by which this pathogen gains access across the intestinal mucosa to macrophages and DCs in the underlying gut-associated lymphoid tissue (Jones and Falkow, 1996). Aggregates of DCs have been shown to be present in isolated lymphoid follicles of the small and large intestine as well as in the Peyer’s patches (Kelsall and Leon, 2005). Recent studies have shown that DCs in the lamina propria have the ability to send dendrites through tight junctions between adjacent epithelial cells and sample bacteria directly from the intestinal lumen (Niess et al., 2005; Rescigno et al., 2001). In this manner DCs, also known to express TLRs (Kadowaki et al., 2001), can undergo direct activation and maturation in response to bacterial TLR ligands such as flagellin (Means et al., 2003). In addition, invasion of IECs with pathogenic Salmonella induces secretion of a variety of cytokines and chemokines from these cells which can also bring about maturation of DCs that enables them to activate T cell responses. These cytokine responses from IECs, as discussed earlier, are produced through activation of TLRs by conserved bacterial molecules (Cario et al., 2000; Hornef et al., 2002). For example, S. typhimurium flagellin stimulates expression and secretion of the CCL20 chemokine in IECs resulting in recruitment of immature DCs expressing the CCL20-specific receptor, CCR6, (Rimoldi et al., 2005a; Sierro et al., 2001). Flagellin also promotes epithelial cell production of the potent neutrophil chemokine IL-8 (Gewirtz et al., 2001a; Gewirtz et al., 2001b). Therefore, the outcome of infection of the gut with Salmonella, and subsequent systemic dissemination of this pathogen might be regulated not only by how IECs or underlying macrophages and DCs respond to the infection but also by a crosstalk between IECs and other cell types. It is known that intestinal homeostasis is regulated by the crosstalk between IECs and DCs (Rimoldi et al., 2005b). Moreover, gut DCs have been shown to
have unique functions. For example, unlike DCs derived from the spleen or from the peripheral blood, mucosal DCs preferentially promote Th2 type of T cell responses during *in vitro* T cell activation (Iwasaki and Kelsall, 1999) and induce secretion of IgA from B cells (Macpherson and Uhr, 2004; Sato *et al.*, 2003). This preferential promotion of Th2 responses by mucosal DCs *in vitro* is taken to be an indicator of a non-inflammatory phenotype in the gut. Microbial infections could disturb this intestinal homeostasis by modulating IEC-DC crosstalk via induction of cytokine secretion from IECs and thereby contribute to inflammation in the gut (Rimoldi *et al.*, 2005b). This kind of a modulation would clearly be influenced by the type of the pathogen that the gut encounters. In this chapter, we have investigated whether direct infection of monocyte-derived human DCs with *S. typhi* or *S. typhimurium* induces different kinds of inflammatory / innate immune responses *in vitro*, and whether soluble mediators produced from IECs infected with these two pathogens can differently modulate responses produced from DCs during infection with *Salmonella*.

### 5.2. RESULTS

#### 5.2.1. Interaction of human monocyte-derived DCs with *S. typhi* or *S. typhimurium* produces similar inflammatory responses

DCs are known to play a vital role in immunity against pathogenic microorganisms including *Salmonella*. In addition to their ability to ingest microorganisms via phagocytosis, these cells also have the capacity to recognize a variety of conserved pathogen associated molecules through membrane bound TLRs (Takeda *et al.*, 2003). Activation of these receptors leads to secretion of cytokines and chemokines from these cells, and also brings about upregulation of adhesion molecules, MHC molecules and co-
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stimulatory molecules on the cell surface (Akira et al., 2001). These activation events result in maturation of DCs into efficient antigen presenting cells that can prime T cell responses (Banchereau and Steinman, 1998; Reis e Sousa, 2001). Depending upon the type of pathogen, DCs can produce different kinds of cytokines which influences the quality and magnitude of T cell responses (Scott et al., 2005). Recent studies have suggested that mucosal DCs expressing TLR-5 might also be important for systemic dissemination of S.typhimurium in mice (Uematsu et al., 2006).

In the present study, DCs were generated from monocytes derived from human peripheral blood, and their identity was confirmed by staining for the DC surface marker CD11c. Cells were then tested for their ability to undergo maturation upon treatment with LPS. Immature (day 6) DCs upregulated expression of MHC-II (HLA-DR) and the costimulatory molecules CD80 and CD86 in response to LPS treatment (Fig. 1), indicating that these cells were functional and capable of undergoing maturation in response to a TLR agonist. To analyze if infection of human DCs with S.typhi or S.typhimurium induces different cellular responses, secretion of various cytokines and expression of various activation markers was analyzed on these cells following infection with these two pathogens. The results showed that infection with both these pathogens resulted in secretion of IL-6, TNF-α, IL-12 and IL-10 from DCs (Fig. 2). The amounts of various cytokines produced from these cells upon infection with S.typhi were comparable with those produced with S.typhimurium. Similarly, the levels of surface expression of MHC class II, co-stimulatory molecules CD80 and CD86 and the DC maturation marker CD83 induced upon infection of DCs with these two pathogens were also comparable (Fig. 3). These results suggested that human monocyte-derived DCs respond efficiently to infection with S.typhi and S.typhimurium, and there are no significant differences in the magnitude of
Figure 1: LPS-matured DCs upregulate expression of cell surface activation markers. (a) Immature DCs were derived from human peripheral blood monocytes by culturing them in presence of rhGM-CSF (1000 U/ml) and rhIL-4 (500 U/ml). Day 6 DCs were stained for the DC cell surface marker CD11c and analyzed by flow cytometry. (b-d) DCs were incubated with S.typhi LPS (1 µg/ml) for 24 h. Untreated and LPS-matured DCs were analyzed for expression of MHC-II (HLA-DR) (b), CD86 (c) and CD83 (d) by flow cytometry.
Figure 2: *S. typhi* and *S. typhimurium* induce similar cytokine responses from human monocyte-derived DCs. DCs were infected with *S. typhi* or *S. typhimurium* at a pathogen to cell ratio of 10:1 for 30 min, followed by incubation in RPMI-10 containing gentamycin (100 μg/ml), rhGM-CSF (1000 U/ml) and rhIL-4 (500 U/ml) for 24 h and supernatants were analyzed for TNF-α, IL-10, IL-6, and IL-12 by ELISA. Error bars, mean ± SD.
Figure 3: DCs infected with *S. typhi* or *S. typhimurium* do not differ in expression of cell surface activation markers. DCs were infected with *S. typhi* and *S. typhimurium* for 30 min and then incubated in RPMI-10 supplemented with gentamycin, rhGM-CSF and rhIL-4 for 24 h. Uninfected and *Salmonella*-infected DCs were stained for surface expression of HLA-DR, costimulatory molecules CD86 and CD80, and the DC maturation marker CD83. Cells were analyzed by flow cytometry.
inflammatory responses induced by these two pathogens. However, it is possible that both the magnitude and quality of innate immune responses produced by DCs following infection with these two pathogens might be different at a later time point during infection. The cytokine responses that were produced in the first 24 h after infection might have been primarily generated through activation of TLR-4 by LPS, a potent activator of DCs (Kaisho et al., 2002; Pulendran et al., 2001; Turnbull et al., 2005), and that may be the reason why differences were not apparent during early stages of infection (Weiss et al., 2004). These responses might change as bacteria establish themselves intracellularly. In this regard, it has been shown that secretion of IL-10 from macrophages following infection with Salmonella is a late event that coincides with induction of molecules of the Salmonella pathogenicity island II (Uchiya et al., 2004). In addition, S. typhimurium is known to downregulate expression of some of its molecules such as flagellin (Cummings et al., 2005; Eriksson et al., 2003) or bring about modifications in other molecules such as LPS (Lu et al., 2003) as it establishes itself within macrophages or DCs. These kind of host-dependent modulations in bacterial effectors have significant effects on inflammatory responses produced during infection with bacterial pathogens (Lu et al., 2003). It is therefore important to study kinetics of expression of cytokines and other cell surface associated and secretory mediators at different stages of infection with S. typhi and S. typhimurium.

5.2.2. Infection with S. typhi but not with S. typhimurium abrogates the ability of IECs to promote a non-inflammatory phenotype in Salmonella-infected DCs

As described previously, the phenotype of mucosal DCs as well as their function is influenced by tissue-specific factors that may be produced by IECs in response to normal
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microbiota or pathogenic microorganisms (Niess and Reinecker, 2006). Both IECs and DCs have the ability to recognize conserved microbial molecules through TLRs and generate a variety of inflammatory responses (Cario et al., 2000; Hornef et al., 2002). DCs also respond to cytokines and chemokines produced by IECs following infection with pathogenic bacteria. Therefore, IECs and DCs are engaged in a constant crosstalk with each other through soluble mediators and possibly through cell to cell contact as well (Niess and Reinecker, 2006), and this crosstalk is known to regulate intestinal homeostasis and in turn inflammation in the gut. Infection of IECs with microbial pathogens could disturb this homeostasis and promote inflammatory responses in the gut.

To investigate if infection of IECs with *S. typhi* and *S. typhimurium* differentially modulates DC functions, IECs were infected with *S. typhi* or *S. typhimurium* and soluble mediators secreted by these cells were tested for their ability to modulate cytokine responses from DCs infected with *Salmonella*. As shown in Fig. 2, infection of human monocyte-derived DCs with these two *Salmonella* species resulted in secretion of a number of cytokines from these cells. Consistent with results reported earlier by Rimoldi et al., conditioning of these DCs with culture supernatant obtained from Caco-2 cells downregulated IL-12 secretion and to a lesser degree secretion of IL-10 in response to infection with *Salmonella* without significantly modifying release of TNF-α (Fig. 4). DCs conditioned with culture supernatant derived from *S. typhimurium*-infected Caco-2 cells similarly showed reduced IL-12 secretion in response to *Salmonella* infection (Fig. 4). In contrast, conditioning with supernatants from *S. typhi*-infected Caco-2 cells did not inhibit secretion of IL-12 from *Salmonella*-infected DCs (Fig. 4). Thus, infection of Caco-2 cells with *S. typhi* suppresses the ability of these cells to modulate IL-12 secretion from infected DCs. These results suggest that infection of IECs with *S. typhi* might disturb intestinal
Figure 4: Soluble mediators from *S.typhi* but not *S.typhimurium*-infected Caco-2 cells promote an inflammatory DC phenotype. Caco-2 cells were left uninfected or infected with *S.typhi* or *S.typhimurium* at a pathogen to cell ratio of 10:1 for 1 h, washed to remove extracellular bacteria and incubated for another 24 h in RPMI-10 containing gentamycin. Immature DCs were conditioned with supernatants from uninfected Caco-2 cells (Uninfected Caco-2 CS) and *S.typhi* or *S.typhimurium* (*S.tym*)-infected Caco-2 cells (Infected Caco-2 CS) for 24 h. Unconditioned DCs were used as controls. Unconditioned and Caco-2-conditioned DCs were infected with *S.typhi* (10 moi) for 30 min, and incubated in RPMI-10 containing gentamycin, rhGM-CSF and rhIL-4 for 24 h. DC supernatants were analyzed for TNF-α, IL-10 and IL-12 by ELISA. Error bars, mean ± SD. The ability of Caco-2 CS to downregulate IL-12 from infected DCs is abrogated upon infection of Caco-2 cells with *S.typhi*. 
homeostasis by activating or inhibiting secretion of soluble mediators from IECs, abrogating their ability to promote a non-inflammatory phenotype in DCs.

5.3. DISCUSSION

Infection of humans with *S. typhi* produces a systemic infection that is characterized by infiltration of mononuclear cells and hypertrophy of the reticuloendothelial system, including the Peyer’s patches, mesenteric lymph nodes, liver, spleen and bone marrow. The first phase of invasion of IECs and dissemination into secondary lymphoid organs is followed by re-entry of bacteria from the gall bladder into the gut. This re-entry is believed to coincide with intestinal inflammation and onset of clinical symptoms (Everest *et al.*, 2001). Unlike *S. typhi*, infection with non-typhoidal *Salmonella* such as *S. typhimurium* results in a self-limiting gastroenteritis that resolves in about a week’s time. Infection with *S. typhimurium* is restricted to the intestine and mesenteric lymph nodes and is associated with a potent inflammatory response that induces transepithelial migration of neutrophils and brings about clearance of bacteria (Criss *et al.*, 2001; McCormick *et al.*, 1995a; McCormick *et al.*, 1993). This kind of inflammatory response is not seen during infection with *S. typhi* (McCormick *et al.*, 1995b). The reasons for these differences are not understood. Recent findings suggest that the outer capsular polysaccharide Vi present in *S. typhi* but not in *S. typhimurium*, might be one of the factors responsible for reduced inflammatory responses with this pathogen. Vi has been shown to target highly conserved prohibitin family of molecules in IECs and downregulate IL-8 secretion during infection of these cells with *S. typhi in vitro* (Sharma and Qadri, 2004). Similar modulation of innate immune responses during infection of macrophages or DCs with *S. typhi* is a distinct possibility. Downregulation of inflammatory responses during the early stages of infection
would promote survival of the pathogen in macrophages and thus, aid in establishment of infection. However, as the disease progresses, inflammatory responses manifest during infection with *S. typhi* as well (Everest *et al.*, 2001). It has been suggested that these responses might be produced *in vivo* by macrophages and T cells which are activated as the infection progresses. However, there is no experimental evidence supporting this proposition. The data presented here suggests that infection with *S. typhi* might disturb immune homeostasis in the gut. It has been previously demonstrated that IECs maintain a non-inflammatory phenotype in mucosal DCs. This is primarily brought about by thymic stromal lymphopoietin (TSLP) secreted by epithelial cells (Rimoldi *et al.*, 2005b). TSLP selectively inhibits secretion of IL-12 from DCs infected with *Salmonella* (Rimoldi *et al.*, 2005b). IL-12 is a key cytokine that promotes Th1 type of T cells which secrete IFN-γ and are proinflammatory in nature (Trinchieri *et al.*, 2003). It has been shown that upon infection with *S. typhimurium*, IECs upregulate expression of TSLP that conditions mucosal DCs to promote a Th2 phenotype in T cells (Rimoldi *et al.*, 2005b). Our results raise the possibility that in contrast to infection with *S. typhimurium*, IECs infected with *S. typhi* might downregulate TSLP or modulate secretion of other mediators leading to an inflammatory DC phenotype. The mechanism of this modulation needs to be investigated. Such a modulation during infection with *S. typhi in vivo* could play a role in producing inflammatory responses that may be dependent upon activation of Th1 type of T cells. Considering that Th1 cells produce IFN-γ which is a key determinant of protective immunity against *Salmonella*, it is possible that this kind of modulation during infection of IECs with *S. typhi* also plays a role in the clearance of the pathogen.

In conclusion, the findings presented in this chapter suggest that through a specific interaction with IECs, *S. typhi* might modulate functions of these cells in a fashion that
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alters immune homeostasis and promotes an inflammatory DC phenotype in the gut. Our results reveal a significant difference between *S.typhi* and *S.typhimurium* in their interaction with human IECs, which might influence the kind of immune response that would be produced during infection with these two pathogens. These findings might have significant implications for *S.typhi* pathogenesis. Future studies should be directed at identifying the cellular mediator that brings about this modulation and deciphering the mechanism of this host-pathogen interaction.