Review Article

Typhi genes expressed during infection or involved in pathogenesis

France Daigle

Department of Microbiology and Immunology, University of Montreal, C.P. 6128 Succursale Centre-Ville, Montréal, Québec, Canada, H3C 3J7

Abstract

Salmonella enterica serovar Typhi (Typhi), the aetiologic agent of typhoid fever, is a human restricted pathogen. Elucidation of the interactions between the infected host and this pathogen is critical to understand infectious diseases but is deterred by a lack of in vivo infection assays, since Typhi uniquely infects humans and there is no suitable animal model. Macrophages can be used as an alternative model, as the ability to survive and replicate within these cells is thought to be one of the major pathogenesis determinants for *Salmonella*. Typhi genes that are expressed within human macrophages have been identified, as well as Typhi immunogenic proteins expressed in humans with typhoid. Known virulence factors of *Salmonella* are expressed during infection of macrophages, such as SPI-2 encoded genes, supporting the validity of the model; however, many genes of unknown functions are also expressed. The importance of these genes should be investigated during future studies aimed at elucidating the intracellular lifestyle of this human-specific pathogen. This review describes Typhi genes expressed during infection or involved in cell interaction.

Key Words: Typhi, *in vivo* gene expression, IVIAT, SCOTS

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Introduction

Understanding Typhi pathogenesis is deterred by a lack of *in vivo* infection assays since Typhi only infects humans; thus there are no suitable animal models available. Because Typhi is restricted to humans, *Salmonella enterica* serovar Typhimurium (Typhimurium), a serovar with a high degree of genome homology (>90%) [1,2], has been used for many years to study typhoid fever pathogenesis using a murine infection model in which Typhimurium causes a systemic infection. This model has been crucial in understanding systemic infections by *Salmonella*. However, it has also been demonstrated that the mouse model does not always reflect the human disease. For example, the ability to disseminate from the bowel and establish extraintestinal niches is promoted by the *spv* locus located on the virulence plasmid in Typhimurium. The absence of the *spv* genes from Typhi is a strong indication that the pathogenesis of typhoid fever is fundamentally different [3]. Moreover, we need to confirm that what we have learned using the murine model with Typhimurium is applicable to Typhi. Many *Salmonella* virulence factors have already been identified and studied, but there are inevitably many more to discover.

Macrophages represent an important host defense mechanism in humans infected with *Salmonella* and the ability to survive and replicate within these cells is thought to be one of the major pathogenesis determinants for *Salmonella* [4,5]. Different molecular approaches have been developed and used to identify *Salmonella* genes expressed during infection. One way to understand molecular mechanisms of pathogenesis is to study the transcriptional response of the bacteria during infection, as virulence factors may be specifically expressed during the course of typhoid infection. Global expression profiling using microarrays can help to define the mechanisms required by the bacteria to cause disease and the host responses required to defeat bacterial infection. However, technical issues currently impede transcriptional profiling of bacterial genes during host infection because of the relatively small number of bacteria present in an infected host, the short half-life of RNA, and the scarcity of polyadenylation on transcripts. Two transcriptomic studies of Typhi have been performed: one that focused on *in vitro* conditions that could be encountered *in vivo*, such as peroxide induced stress [6], and one during infection of human macrophages [7]. Proteomic profiling is another approach that has been

used to obtain information regarding bacterial metabolism or mechanisms used to survive within various cell types by identifying proteins produced within host cells [8,9]. Proteomics methods have been based on the use of different mass spectrometry-based methods to identify bacterial proteins produced within host cells. A proteomics analysis of Typhi grown in low pH, low magnesium minimal media (MgM or LPM) was recently reported [10]. MgM is designed to approximate the phagosome of infected macrophages and is known to induce expression of SPI-2 virulence genes and other genes related to virulence and intramacrophage survival [11]. Another strategy that has recently been developed identifies bacterial antigens that are immunogenic and produced during infection, a technique called *in vivo* induced antigen technology (IVIAT) [12]. This technique was successfully used to identify Typhi proteins produced in typhoid patients [13].

In this review, *in vivo* gene expression of Typhi is described, focusing mainly on Typhi genes expressed within macrophages.

Salmonella pathogenicity islands (SPI)

SPIs are insertions of large regions of DNA containing virulence genes, located on the bacterial chromosome. These gene blocks are often inserted near a tRNA gene and generally display distinct codon usage and a different overall base composition from the core bacterial chromosome, suggesting that they were acquired from a foreign source. Thus far, 15 SPIs have been identified in Typhi [1,14]. SPI-1 and SPI-2, which are present in all *S. enterica* serovars, represent 2 major pathogenesis determinants that encode type III secretion systems (T3SS). SPI-1 and SPI-2 T3SS have distinct roles in *Salmonella* pathogenesis. SPI-1 effectors are injected into host cells via the T3SS and are required for invasion of epithelial cells [15], whereas SPI-2 contributes to *Salmonella* survival inside macrophages [16, 17]. Although these systems are important and even crucial for Typhimurium, very little information is available concerning Typhi.

SPI-1. Many Typhi genes involved in invasion identified so far are homologous genes present in Typhimurium, including SPI-1 genes (*invC, invA, invE, invG, prgH, iagAB, sipEBCDA*) [18-23] (Table 1). There should be distinct invasion genes that remain unidentified as Typhi adheres, invades, and migrates through human epithelial cells better than Typhimurium [20,24]. Moreover, optimal adherence occurs at high osmolarity, when the Vi capsule is produced at its lowest levels [20,25]. Some SPI-1 genes are up-regulated following uptake by macrophages, but most genes are not differentially expressed when compared to bacteria present in the supernatant of macrophages [7]. This finding may be different if another growth condition is used as the comparator. Thus, as for Typhimurium, a key role for SPI-1 T3SS in invasion is predicted for Typhi.

SPI-2. The SPI-2 locus of *S. enterica* is 40 kb in size and is divided into two functional entities. A portion of 25 kb, important for virulence, contains the T3SS and several translocated effectors. In contrast, the 15-kb portion encodes the tetrathionate reduction (*ttr*) system and proteins of unknown function and was not required for virulence [26]. Although a crucial role in virulence for SPI-2 in Typhimurium has been demonstrated and its importance has been well established, the data concerning Typhi SPI-2 genes are almost nonexistent. A Typhi strain bearing a double mutation in *ssaV* (a SPI-2 gene) and in *aroC* (aromatic biosynthesis) was previously shown to survive less efficiently in human macrophages compared to the wild-type parent strain [27]. In human volunteers, this doubly mutated strain was attenuated [28] (Table 1). Within human macrophages, many of the Typhi SPI-2 genes that belong to the 25 kb locus (18 out of 31) are up-regulated following bacterial uptake, and the majority of SPI-2 encoded genes are up-regulated 2 h post-infection (29 out of 31). Most of the SPI-2 encoding genes are also up-regulated until 24 hours post-infection (26 out of 31) [7]. The Typhi SPI-2 genes located on the 15 kb locus are not differentially expressed. Moreover, Typhi may not use the *ttr* system as *ttrS* is a pseudogene in CT18 [1].

SPI-7. SPI-7 is a large 134 kb segment which is absent from Typhimurium and encodes the Vi antigen as well as the Type IV pili (see adhesins) and the SopE prophage. The virulence of Typhi is associated with the presence of the Vi antigen, which is needed for Typhi to survive inside phagocytes and necessary for serum resistance, a characteristic required for systemic dissemination [29-31]. The Vi polysaccharidic capsule is encoded by the *viaB* locus, which is composed of 11 genes and contains two regions: one involved in biosynthesis, including *tviA-E*, and the other required for translocation of the polysaccharide to the cell surface, *vexA-E* [32]. The cDNA corresponding to the *tviB* gene has been captured and cloned from human macrophages 2 hours post infection but is not detected 24 hours post-infection [33]. A transcriptomic study of Typhi genes in macrophages did not demonstrate up-

regulation of the *viaB* locus; however, the *tviABCD* region was not present on the microarray and expression of these genes could not be assessed [7]. However, a Typhi strain harboring a deletion in the *viaB* locus was shown to be highly invasive, which can be explained by the production of the SPI-1 T3SS [23]. Similarly, by using a *tviB* mutant, the presence of the Vi capsule has been shown to inhibit adhesion as well as invasion of epithelial cells [34] (Table 1).

Table 1. Typhi genes involved in pathogenesis.

Class	Gene or ORF	Strain	Phenotype/ Expression	Ref.
SPI-1	*invC (spaI)*	ISP1820	Non invasive Henle-407	[18]
	invA	ISP1820, GIFU10007	Non invasive on INT-407	[20, 23]
	invE	ISP1820	Non invasive on INT-407	[20]
	invG	ISP1820	Non invasive on INT-407	[19]
	prgH	ISP1820	Non invasive on INT-407	[19]
	iagAB (hilA)	Ty2	Non invasive on HeLa	[62]
	sipEBCDA	Ty2	Non invasive on HeLa	[21]
SPI-2	*ssaV**	Ty2	Less survival in U937 macrophage; attenuated in human	[27, 28]
	ssaTS, yscR, ssaN, ssaJ, sscB, sseED, sscA, sseB, ssaED, spiA, ssrA, ssaV	ISP1820	Up in THP-1	[7]
Class	**Gene or ORF**	**Strain**	**Phenotype/ Expression**	**Ref.**
SPI-3	*mgtC, mgtB*	ISP1820	Up in THP-1	[7]
SPI-5	*pipD*	ISP1820	Up in THP-1	[7]
SPI-6	*safE, safB*	ISP1820	Up in THP-1	[7]
SPI-7	STY4633	ISP1820	Up in THP-1	[7]
	tviB	ISP1820	Up 2h pi in THP-1	[33]
		TY2	More invasive on INT-407	[34]
	viaB locus	GIFU10007	More invasive on INT-407	[23]
SPI-10	*prpZ*	Ty2	Less survival in THP-1	[35]
SPI-11	*pagC*		Detected in human serum	[13]
		ISP1820	Up in THP-1	[7]
	pagD, envE	ISP1820	Up in THP-1	[7]
Adhesion	*tcfB*		Detected in human serum	[13]
	stbD		Detected in human serum	[13]
	csgGFE		Detected in human serum	[13]
	stgABCD	ISP1820	Less adherent to INT-407; higher phagocytosis by THP-1	[39]
	pil	J341	Less entry in INT-407, and THP-1	[37, 38]
	fim	GIFU10007	More invasive on INT-407	[36]
Anti-microbial resistance	*acrA*		Detected in human serum	[13]
	pspCDE		Detected in human serum	[13]
		ISP1820	Up in THP-1	[7]
	phoP, pmrF, ugtL, pqaB, pgtE, mig-14, somA	ISP1820	Up in THP-1	[7]
	phoP	TY2	Restricted in U937; Less invasive to HT-29	[43, 59]
	mliC (ydhA)	ISP1820	Lower survival in THP-1	[33]
Motility	*fliA, flhDC*	ISP1820	Less invasion of Henle-407; Less cytotoxic on J774.A1	[18]
	fla-	GIFU10007	Non invasive on HeLa	[61]
Sigma factor	*rpoS*	GIFU10007	Less cytotoxic on THP-1	[58]
		Ty2	Less invasive on HT-29	[57]

*also *aroC*-.

Other SPIs. The role of genes that belong to other pathogenicity islands in Typhi pathogenesis has not been investigated in depth yet. The magnesium transport system *mgtBC* located on SPI-3 is strongly induced intracellularly by Typhi during infection of macrophages, as well as *pipD* located on SPI-5, and *pagC*, *pagD*, and *envE* of SPI-11 [7]. PagC is produced in typhoid patients [13]. On SPI-10, the *prpZ* locus encoding for proteins with homology to eukaryotic-type Ser/Thr protein phosphatase and kinases has been found to promote survival in macrophages [35] (Table 1).

Adhesins

The genome of serovar Typhi contains 13 putative operons corresponding to fimbrial gene sequences termed *bcf, csg (agf), fim, saf, sef, sta, stb, stc, std, ste, stg, sth* and *tcf*, and *pil*, the type IV pili [1]. Five of these operons, *sef, sta, ste, stg*, and *tcf*, and the Type IV pili are not detected in serovar Typhimurium [1] and

may be important for Typhi host specificity. In typhoid fever patients, antibody to 3 fimbrial systems, TcfB, StbD, and CsgEFG, has been detected [13]. The *csg* operon is also up-regulated 2 hours post infection inside macrophages [7]. A Typhi strain carrying a deletion of the *fim* genes, encoding for type 1 fimbriae, is more invasive than the wild-type strain [36]. In absence of Type 1 fimbriae, different bacterial surface proteins may become available to interact with host cells, causing a higher level of invasion. The *pil* genes encoding for type IV pili facilitate Typhi entry into human intestinal epithelial cells and macrophages [37,38]. The deletion of *stg* reduced adherence of Typhi to epithelial cells but increased uptake by human macrophages, although survival inside macrophages was similar to the wild-type parent [39] (Table 1).

Antimicrobial resistance

An important host defense mechanism involves production of antimicrobial peptides. Responses to antimicrobial peptides in *Salmonella* involve the *phoPQ* two-component system regulator, the *pmr* operon [40], *ugtL* [41], *pagP* [42], *pqaB* [43], *pgtE* [44], *virK* and its homologue *somA* (*ybjX*) [45] and *mig-14* [46, 47]. These genes are up-regulated by Typhi in human macrophages [7]. The PhoPQ system has also been shown to regulate hundred of genes in *Salmonella*, reviewed in [48]. A *phoP* mutant was shown to be restricted in net growth in U937 macrophages [43] and the live attenuated vaccine strain Ty800 harbours a *phoP* mutation [49] (Table 1).

Lysosyme is another component produced by the host to eliminate the bacterial invader by attacking the bacterial cell wall, but some bacteria produce lysosyme inhibitors to evade antibacterial enzymes. A novel family of lysozyme inhibitors was recently discovered, *mliC* (*ydhA*) (membrane bound lysosyme inhibitor of c-type lysozyme) [50] and seem to promote macrophage survival of Typhi [33]. The phage-shock-protein (Psp) system responds to stresses and may be involved in antimicrobial resistance [51]. PspC, PspD and PspE were identified by IVIAT during human infection with Typhi [13] and are also up-regulated in human macrophages [7] (Table 1).

Metal transport

It has been shown that a Typhi mutant defective for enterochelin synthesis and transport has a lower ability to enter and proliferate in epithelial cells and macrophages [52-54]. Some proteins involved in heavy metal transport, MerP and STY0909, were identified by IVIAT during human infection with Typhi [13]. However, during infection of human macrophages, Typhi genes involved in iron acquisition and transport (such as *fes, fhu, feo, ent, iro*) are down-regulated intracellularly [7]. It is possible that these genes are already up-regulated in the supernatant of infected cells or the conditions that were used as a comparator. Alternatively, it may be a bias of the cultured THP-1 model as intracellular pathogens such as *Mycobacterium tuberculosis* and Typhi do not seem to face iron limiting conditions in these cells; their transcriptional profiles did not correspond with a predicted low-iron environment [7,55].

Other genes

The sigma factor RpoS is a global stationary phase regulator, controlling expression of many virulence associated systems, including Vi synthesis [56], and is required for virulence of *Salmonella* [57]. A mutation in *rpoS* renders Typhi less cytotoxic to THP-1 macrophages and although the mutant survives similarly to the wild-type strain [58], the mutant was less invasive on epithelial cells [59] (Table 1). Transcription of *rpoS* was shown to increase in macrophages [7, 58]. Moreover, Ty2 and the live oral vaccine Ty21a strains are *rpoS* mutants [60].

Typhi flagellar mutants (*flhDC* or *fliA*) are deficient in cell invasion, and result in a reduction of SPI-1 gene expression, which is more pronounced in Typhi than Typhimurium [18]. Macrophage cytotoxicity is also reduced in flagellar mutants [18]. The motility defect cannot be restored by centrifugation as observed with Typhimurium [61].

Conclusion

Host-pathogen interactions are very complex and considerable effort is required for their elucidation. Studying interactions between the infected host and Typhi should improve our understanding of typhoid fever. Typhi has developed remarkable persistence mechanisms within the host that help ensure its survival and transmission. However, data on human typhoid collected by using modern immunological and molecular techniques are scarce since Typhi uniquely infects humans, and there are no suitable animal models available. As survival within macrophages is an essential step for *Salmonella* pathogenesis, macrophages represent a useful model to study Typhi. Typhi gene expression during infection was monitored in human macrophages. In effect, the transcriptome of Typhi from infected macrophages at 2 hours, 8 hours,

and 24 hours post-infection has been obtained [7]. Interestingly, 117 genes are up-regulated at all intracellular time points. Of these, 32 genes belong to SPIs or have been previously associated with pathogenicity, including 17 genes from SPI-2, involved in intracellular survival. Twenty genes that belong to the membrane lipoproteins and porins class were identified, suggesting important adaptations and modifications at the outer membrane level. Many genes (30) encoding for hypothetical or unknown proteins were identified and await further investigation to determine their possible roles as novel virulence factors. Similarly, 19 of the constitutively up-regulated genes in macrophages are absent in Typhimurium, suggesting such Typhi-specific genes may be involved in survival in macrophages. Because of a lack of accumulated data, it may be too premature to compare gene expression between Typhi and Typhimurium. Moreover, the model used is often different. However, Typhi is less adherent, invasive and cytotoxic than Typhimurium [4, 34] suggesting that many differences are left to be discovered.

In order to circumvent the limitations associated with *in vitro* models, proteins that are immunogenic and expressed uniquely in humans with typhoid were identified using IVIAT [13]. Of the 30 identified antigens encoded on the chromosome, 16 were also up-regulated at least at one time point during infection of macrophages. It should be stressed that no single approach will provide all of the information necessary for the desired level of understanding of the infectious processes. Each approach, sometimes in combination with one or another approach, will provide superior results in some cases but not in others. Thus, it will be the collective efforts of many investigators, using the diversity of established as well as novel approaches, that we will achieve our ultimate goal to fully understand the mechanisms of *Salmonella* persistence, transmission, infectivity and pathogenesis. Elucidating the bacterial genes expressed in the host and those underlying typhoid pathogenesis should lead to the development of new strategies including novel anti-bacterial treatments and identification of novel vaccine candidates to control the disease.

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References

1. Parkhill J *et al*. (2001) Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. Nature 413: 848-852.
2. McClelland M *et al*. (2001) Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature 413: 852-856.
3. Fierer J, and Guiney DG (2001) Diverse virulence traits underlying different clinical outcomes of *Salmonella* infection. J Clin Invest 107: 775-780.
4. Schwan W, Huang X, Hu L, and Kopecko D (2000) Differential bacterial survival, replication, and apoptosis-inducing ability of *Salmonella* serovars within human and murine macrophages. Infect Immun 68: 1005-1013.
5. Fields PI, Swanson RV, Haidaris CG, and Heffron F (1986) Mutants of *Salmonella* typhimurium that cannot survive within the macrophage are avirulent. Proc Natl Acad Sci (USA) 83: 5189-5193.
6. Porwollik S, Frye J, Florea LD, Blackmer F, and McClelland M (2003) A non-redundant microarray of genes for two related bacteria. Nucleic Acids Res 31: 1869-1876.
7. Faucher SP, Porwollik S, Dozois CM, McClelland M, and Daigle F (2006) Transcriptome of *Salmonella enterica* serovar Typhi within macrophages revealed through the selective capture of transcribed sequences. Proc Natl Acad Sci (USA) 103: 1906-1911.
8. Burns-Keliher LL, Portteus A, and Curtiss III R (1997) Specific detection of *Salmonella* typhimurium proteins synthesized intracellularly. J Bacteriol 179: 3604-3612.
9. Adkins JN *et al*. (2006) Analysis of the *Salmonella typhimurium* proteome through environmental response toward infectious conditions. Mol Cell Proteomics 5: 1450-1461.
10. Ansong C *et al*. (2008) Proteomics analysis of the causative agent of typhoid fever. J Proteome Res 7: 546-557.
11. Coombes BK, Brown NF, Valdez Y, Brumell JH, and Finlay BB (2004) Expression and secretion of *Salmonella* pathogenicity island-2 virulence genes in response to acidification exhibit differential requirements of a functional type III secretion apparatus and SsaL. J Biol Chem 279: 49804-49815.
12. Rollins SM *et al*. (2005) In vivo induced antigen technology (IVIAT). Cell Microbiol 7: 1-9.
13. Harris JB *et al*. (2006) Identification of *in vivo*-induced bacterial protein antigens during human infection with *Salmonella enterica* serovar Typhi. Infect Immun 74: 5161-5168.
14. Vernikos GS, and Parkhill J (2006) Interpolated variable order motifs for identification of horizontally acquired DNA: revisiting the *Salmonella* pathogenicity islands. Bioinformatics 22: 2196-2203.
15. Galan JE (1999) Interaction of *Salmonella* with host cells through the centisome 63 type III secretion system. Curr Opin Microbiol 2: 46-50.
16. Cirillo DM, Valdivia RH, Monack DM, and Falkow S (1998) Macrophage-dependent induction of the *Salmonella* pathogenicity island 2 type III secretion system and its role in intracellular survival. Mol Microbiol 30: 175-188.
17. Hensel M *et al*. (1998) Genes encoding putative effector proteins of the type III secretion system of *Salmonella* pathogenicity island 2 are required for bacterial virulence and proliferation in macrophages. Mol Microbiol 30: 163-174.
18. Eichelberg K, and Galan JE (2000) The flagellar sigma factor FliA (sigma(28)) regulates the expression of *Salmonella* genes

associated with the centisome 63 type III secretion system. Infect Immun 68: 2735-2743.
19. Leclerc GJ, Tartera C, and Metcalf ES (1998) Environmental regulation of *Salmonella typhi* invasion-defective mutants. Infect Immun 66: 682-691.
20. Weinstein DL, O'Neill BL, Hone DM, and Metcalf ES (1998) Differential early interactions between *Salmonella enterica* serovar Typhi and two other pathogenic *Salmonella* serovars with intestinal epithelial cells. Infect Immun 66: 2310-2318.
21. Hermant D, Menard R, Arricau N, Parsot C, and Popoff MY (1995) Functional conservation of the *Salmonella* and *Shigella* effectors of entry into epithelial cells. Mol Microbiol 17: 781-789.
22. Elsinghorst EA, Baron LS, and Kopecko DJ (1989) Penetration of human intestinal epithelial cells by *Salmonella*: molecular cloning and expression of *Salmonella typhi* invasion determinants in *Escherichia coli*. Proc Natl Acad Sci (USA) 86: 5173-5177.
23. Zhao L *et al*. (2001) Vi-Suppressed wild strain *Salmonella typhi* cultured in high osmolarity is hyperinvasive toward epithelial cells and destructive of Peyer's patches. Microbiol Immunol 45: 149-158.
24. Kops SK, Lowe DK, Bement WM, and West AB (1996) Migration of *Salmonella typhi* through intestinal epithelial monolayers: an in vitro study. Microbiol Immunol 40: 799-811.
25. Tartera C, and Metcalf ES (1993) Osmolarity and growth phase overlap in regulation of *Salmonella typhi* adherence to and invasion of human intestinal cells. Infect Immun 61: 3084-3089.
26. Hensel M, Nikolaus T, and Egelseer C (1999) Molecular and functional analysis indicates a mosaic structure of *Salmonella* pathogenicity island 2. Mol Microbiol 31: 489-498.
27. Khan SA *et al*. (2003) *Salmonella typhi* and *S. typhimurium* derivatives harbouring deletions in aromatic biosynthesis and *Salmonella* Pathogenicity Island-2 (SPI-2) genes as vaccines and vectors. Vaccine 21: 538-548.
28. Hindle Z *et al*. (2002) Characterization of *Salmonella enterica* derivatives harboring defined *aroC* and *Salmonella* pathogenicity island 2 type III secretion system (*ssaV*) mutations by immunization of healthy volunteers. Infect Immun 70: 3457-3467.
29. Hornick RB *et al*. (1970) Typhoid fever: pathogenesis and immunologic control. N Engl J Med 283: 686-691.
30. Looney RJ, and Steigbigel RT (1986) Role of the Vi antigen of *Salmonella typhi* in resistance to host defense in vitro. J Lab Clin Med 108: 506-516.
31. Hirose K *et al*. (1997) Survival of Vi-capsulated and Vi-deleted *Salmonella typhi* strains in cultured macrophage expressing different levels of CD14 antigen. FEMS Microbiol Lett 147: 259-265.
32. Virlogeux I, Waxin H, Ecobichon C, and Popoff MY (1995) Role of the *viaB* locus in synthesis, transport and expression of *Salmonella typhi* Vi antigen. Microbiology 141: 3039-3047.
33. Daigle F, Graham JE, and Curtiss R, 3rd (2001) Identification of *Salmonella typhi* genes expressed within macrophages by selective capture of transcribed sequences (SCOTS). Mol Microbiol 41: 1211-1222.
34. Bishop A *et al*. (2008) Interaction of *Salmonella enterica* serovar Typhi with cultured epithelial cells: roles of surface structures in adhesion and invasion. Microbiology 154: 1914-1926.
35. Faucher SP, Viau C, Gros PP, Daigle F, and Le Moual H (2008) The *prpZ* gene cluster encoding eukaryotic-type Ser/Thr protein kinases and phosphatases is repressed by oxidative stress and involved in *Salmonella enterica* serovar Typhi survival in human macrophages. FEMS Microbiol Lett 281:160-166.
36. Miyake M *et al*. (1998) Vi-deficient and nonfimbriated mutants of *Salmonella typhi* agglutinate human blood type antigens and are hyperinvasive. FEMS Microbiol Lett 161: 75-82.
37. Zhang XL *et al*. (2000) *Salmonella enterica* serovar *typhi* uses type IVB pili to enter human intestinal epithelial cells. Infect Immun 68: 3067-3073.
38. Pan Q *et al*. (2005) Aptamers that preferentially bind type IVB pili and inhibit human monocytic-cell invasion by *Salmonella enterica* serovar *typhi.* Antimicrob Agents Chemother 49: 4052-4060.
39. Forest C *et al*. (2007) Contribution of the *stg* fimbrial operon of *Salmonella enterica* serovar Typhi during interaction with human cells. Infect Immun 75: 5264-5271.
40. Gunn JS, Ryan SS, Van Velkinburgh JC, Ernst RK, and Miller SI (2000) Genetic and functional analysis of a PmrA-PmrB-regulated locus necessary for lipopolysaccharide modification, antimicrobial peptide resistance, and oral virulence of *Salmonella enterica* serovar *typhimurium*. Infect Immun 68: 6139-6146.
41. Shi Y, Cromie MJ, Hsu FF, Turk J, and Groisman EA (2004) *PhoP*-regulated *Salmonella* resistance to the antimicrobial peptides magainin 2 and polymyxin B. Mol Microbiol 53: 229-241.
42. Guo L *et al*. (1998) Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides. Cell 95: 189-198.
43. Baker SJ, Gunn JS, and Morona R (1999) The *Salmonella* typhi melittin resistance gene *pqaB* affects intracellular growth in PMA-differentiated U937 cells, polymyxin B resistance and lipopolysaccharide. Microbiology 145: 367-378.
44. Guina T, Yi EC, Wang H, Hackett M, and Miller SI (2000) A PhoP-regulated outer membrane protease of *Salmonella enterica* serovar *typhimurium* promotes resistance to alpha-helical antimicrobial peptides. J Bacteriol 182: 4077-4086.
45. Detweiler CS, Monack DM, Brodsky IE, Mathew H, and Falkow S (2003) *virK*, *somA* and *rcsC* are important for systemic *Salmonella enterica* serovar Typhimurium infection and cationic peptide resistance. Mol Microbiol 48: 385-400.
46. Brodsky IE, Ernst RK, Miller SI, and Falkow S (2002) *mig*-14 is a *Salmonella* gene that plays a role in bacterial resistance to antimicrobial peptides. J Bacteriol 184: 3203-3213.
47. Brodsky IE, Ghori N, Falkow S, and Monack D (2005) *Mig*-14 is an inner membrane-associated protein that promotes *Salmonella typhimurium* resistance to CRAMP, survival within activated macrophages and persistent infection. Mol Microbiol 55: 954-972.
48. Prost LR, and Miller SI (2008) The *Salmonellae* PhoQ sensor: mechanisms of detection of phagosome signals. Cell Microbiol 10: 576-582.
49. Hohmann EL, Oletta CA, Killeen KP, and Miller SI (1996) *phoP*/*phoQ*-deleted *Salmonella typhi* (Ty800) is a safe and immunogenic single-dose typhoid fever vaccine in volunteers. J Infect Dis 173: 1408-1414.
50. Callewaert L *et al*. (2008) A new family of lysozyme inhibitors contributing to lysozyme tolerance in gram-negative bacteria. PLoS Pathog 4: e1000019.
51. Darwin AJ (2005) The phage-shock-protein response. Mol Microbiol 57: 621-628.
52. Contreras I, Toro CS, Troncoso G, and Mora GC (1997) *Salmonella typhi* mutants defective in anaerobic respiration are

impaired in their ability to replicate within epithelial cells. Microbiology 143: 2665-2672.
53. Furman M, Fica A, Saxena M, Di Fabio JL, and Cabello FC (1994) *Salmonella typhi* iron uptake mutants are attenuated in mice. Infect Immun 62: 4091-4094.
54. Gorbacheva VY, Faundez G, Godfrey HP, and Cabello FC (2001) Restricted growth of *ent*(-) and *tonB* mutants of *Salmonella enterica* serovar Typhi in human Mono Mac 6 monocytic cells. FEMS Microbiol Lett 196: 7-11.
55. Fontan P, Aris V, Ghanny S, Soteropoulos P, and Smith I (2008) Global transcriptional profile of *Mycobacterium tuberculosis* during THP-1 human macrophage infection. Infect Immun 76: 717-725.
56. Santander J, Wanda SY, Nickerson CA, and Curtiss R, 3rd (2007) Role of RpoS in fine-tuning the synthesis of Vi capsular polysaccharide in *Salmonella enterica* serotype Typhi. Infect Immun 75: 1382-1392.
57. Fang FC *et al*. (1992) The alternative sigma factor *katF* (*rpoS*) regulates *Salmonella* virulence. Proc Natl Acad Sci (USA) 89: 11978-11982.
58. Khan AQ *et al*. (1998) *Salmonella typhi rpoS* mutant is less cytotoxic than the parent strain but survives inside resting THP-1 macrophages. FEMS Microbiol Lett 161: 201-208.
59. Lee HY *et al*. (2007) Evaluation of *phoP* and *rpoS* mutants of *Salmonella enterica* serovar Typhi as attenuated typhoid vaccine candidates: virulence and protective immune responses in intranasally immunized mice. FEMS Immunol Med Microbiol 51: 310-318.
60. Robbe-Saule V, and Norel F (1999) The *rpoS* mutant allele of *Salmonella* typhi Ty2 is identical to that of the live typhoid vaccine Ty21a. FEMS Microbiol Lett 170:141-143.
61. Liu SL, Ezaki T, Miura H, Matsui K, and Yabuuchi E (1988) Intact motility as a *Salmonella typhi* invasion-related factor. Infect Immun 56: 1967-1973.
62. Miras I, Hermant D, Arricau N, and Popoff MY (1995) Nucleotide sequence of *iagA* and *iagB* genes involved in invasion of HeLa cells by *Salmonella enterica* subsp. *enterica* ser. Typhi. Res Microbiol 146: 17-20.

Corresponding Author: France Daigle, Department of Microbiology and Immunology, University of Montreal, C.P. 6128 Succursale Centre-Ville, Montréal, Québec, Canada, H3C 3J7
Tel. (514) 343-7396; Fax (514) 343-5701
Email: france.daigle@umontreal.ca

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