The Vi antigen of Salmonella typhi

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Bull. Inst. Pasteur
1996, 94, 237-250

The Vi antigen of *Salmonella typhi*

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Key-words: Salmonella typhi, Vi antigen; Biogenesis, Virulence, Vaccine; Review.

Introduction

A large number of pathogenic bacteria implicated in serious invasive infections for humans (including septicaemia, meningitis, pneumonia, etc.) are encapsulated (*Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae* type b...). These capsules, in most cases made of polysaccharides, are the most external structure of the cell and therefore play a critical part in the bacteria-environment interactions by being an important virulence factor for the host and/or a bacterial protection factor. Moreover, capsular polysaccharides are, with few exceptions, immunogenic in man and able to induce antibodies involved in the neutralization of the capsulated bacteria (Jennings, 1990; Robbins *et al.*, 1995). These characteristics and the fact that they are non-toxic and free of other deleterious effects associated with whole organism vaccines enhance the interest in using capsular polysaccharides as molecular vaccines (Robbins, 1978; Jennings, 1983).

Received October 11, 1996.

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The Vi antigen is a capsular polysaccharide found mainly in *Salmonella typhi* and *Salmonella paratyphi* C, two serotypes of *Salmonella* which are responsible for severe infections in humans (Felix and Pitt, 1936; Baker *et al.*, 1959). When Felix and Pitt (1934) discovered this surface structure covering *S. typhi*, they named it "Vi antigen", for *vi*rulence, based on its ability to enhance *S. typhi* virulence in mice and to induce an immune response in rabbits. Since then, research on Vi antigen has been focused on this potential role in *S. typhi* virulence and the development of vaccines against typhoid fever constituted of Vi antigen. In this review, we first present information on the characteristics and the biogenesis of the Vi antigen of *S. typhi*. Secondly, we present knowledge on its potential role in the virulence of *S. typhi* and on its protective activity.

Characterization of Vi antigen

The Vi antigen is a linear homopolymer of α-1,4-linked *N*-acetyl galactosaminuronic acid, variably *O*-acetylated at the C-3 position (fig. 1)

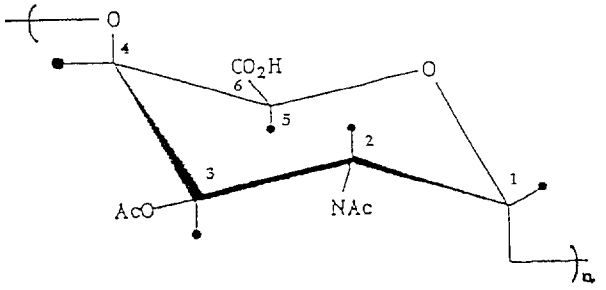

Fig. 1. Repeating structure of the *S. typhi* Vi antigen (adapted from Stone and Szu, 1988).

The Vi antigen is a homopolymer of *N*-acetylgalactosaminuronic acid linked in α-(1,4) and variably *O*-acetylated at the C3 position.

NAc = *N*-acetyl group; AcO = *O*-acetyl group; • = hydrogen.

(Heyns and Kiessling, 1967; Daniels *et al.*, 1989). The assembly of these monomers constitutes a very high molecular weight polysaccharide (typically, more than 10^6 daltons). Its expression is not thermoregulated and after 24 h of culture, more than 98% of the synthesized Vi antigen is released in the culture supernatant of *S. typhi* (Daniels *et al.*, 1989; Tsang and Wong, 1989). The remaining 2% of Vi antigen is cell-associated and coexpressed with the O9 antigen of *S. typhi* (Daniels *et al.*, 1989). Based on these structural and biochemical properties, the Vi antigen of *S. typhi* could therefore be considered as a group I polysaccharide (Jann and Jann, 1990).

Expression of Vi antigen is controlled by three widely separated loci, *viaA*, *viaB* and *ompB* (Johnson *et al.*, 1965; Pickard *et al.*, 1994). The *viaA* locus, located at 43 min on the chromosome of *S. typhi*, is commonly found in enteric bacteria (Johnson and Baron, 1969) and is allelic to *rcsB*, a positive regulator of capsule synthesis in *E. coli* (Houng *et al.*, 1992; Gottesman and Stout, 1991). Cloning, sequencing and complementation experiments indicated that, indeed, the *viaA* locus of *S. typhi* encompasses the *rcsB* but also *rcsC* gene (Virlogeux *et al.*, 1996a). The predicted amino acid sequences of the RcsB protein and of the C-terminal region of RcsC show a high degree of similarity to their *E. coli* homologs (98% and 86%, respectively). Moreover, a possible helix turn helix DNA binding motif has been identified in the C-terminal domain of RcsB (Virlogeux *et al.*, 1996a).

In contrast, the *viaB* locus, which maps at 92 min on the chromosome of *S. typhi* (Johnson and Baron, 1969), is specific to Vi-expressing strains and consists of the structural genes required for Vi antigen expression (Hashimoto *et al.*, 1991; Kolyva *et al.*, 1992; Hashimoto *et al.*, 1993; Waxin *et al.*, 1993; Virlogeux *et al.*, 1995). Acquisition of the *viaB* region by *E. coli* results in a Vi-positive phenotype (Johnson and Baron, 1969; Hashimoto *et al.*, 1991; Kolyva *et*

ORF = open reading frame.

al., 1992). This locus is located on a 13.5-kb chromosomal DNA fragment and contains 11 open reading frames (ORF), designated *tviA-E* (*t*yphi *Vi*), *vexA-E* (*V*i antigen *ex*port) and ORF11, all being transcribed in the same orientation from *tviA* to ORF11 (fig. 2) (Hashimoto *et al.*, 1993; Waxin *et al.*, 1993). At the least, *tviA*, *tviB* and *tviC* genes are cotranscribed (Virlogeux *et al.*, 1995; Hashimoto *et al.*, 1996; Virlogeux *et al.*, 1996a). The transcriptional start site of this operonic structure has been identified 131 bp upstream of the ATG codon of the *tviA* gene (Virlogeux *et al.*, 1996a). Genetic determinants of the *viaB* locus encode polypeptides with a molecular mass between 21 kDa and 80 kDa (fig. 2) (Kolyva *et al.*, 1992; Hashimoto *et al.*, 1993). The ORF11 product with a predicted molecular mass of 7 kDa was not identified (Hashimoto *et al.*, 1993).

The *ompB* locus, comprising the *ompR* and *envZ* genes, maps at 74 min on the chromosome of *S. typhimurium* (Sanderson and Hurley, 1987). It was shown to be involved in the expression of Vi antigen too (Pickard *et al.*, 1994). Indeed, *S. typhi ompR* mutants not only displayed a marked decrease in OmpC and OmpF porin expression as expected (Puente *et al.*, 1991), but also showed a loss of Vi antigen synthesis.

Biogenesis of Vi antigen

General principles of capsule biogenesis

The biogenesis of capsular polysaccharides was mainly studied for the group II polysaccharides. These polysaccharides which are of lower molecular weight (typically less than 50 kD) than group I polysaccharides, are not synthesized at temperatures below 20°C (Jann and Jann, 1990). This temperature control of their expression was used to great advantage to study their biogenesis.

The expression of capsules by the bacterial cell is a very complex process, passing through stages which are associated with different cellular compartments. The first step in capsular biogenesis, which occurs in the cytoplasmic compartment, is the synthesis of sugar constituents and their activation as nucleotide sugars. The subsequent polymerization is catalysed by an inner membrane transferase complex and proceeds processively at the non-reducing terminus by the addition of activated monomers to the growing polysaccharide chain (Troy and McCloskey, 1979; Masson and Holbein, 1983; Vimr and Steebergen, 1993). Generally, polymerization is believed to involve a lipid carrier on which monosaccharides or oligosaccharides are assembled (Boulnois and Roberts, 1990). The polysac-

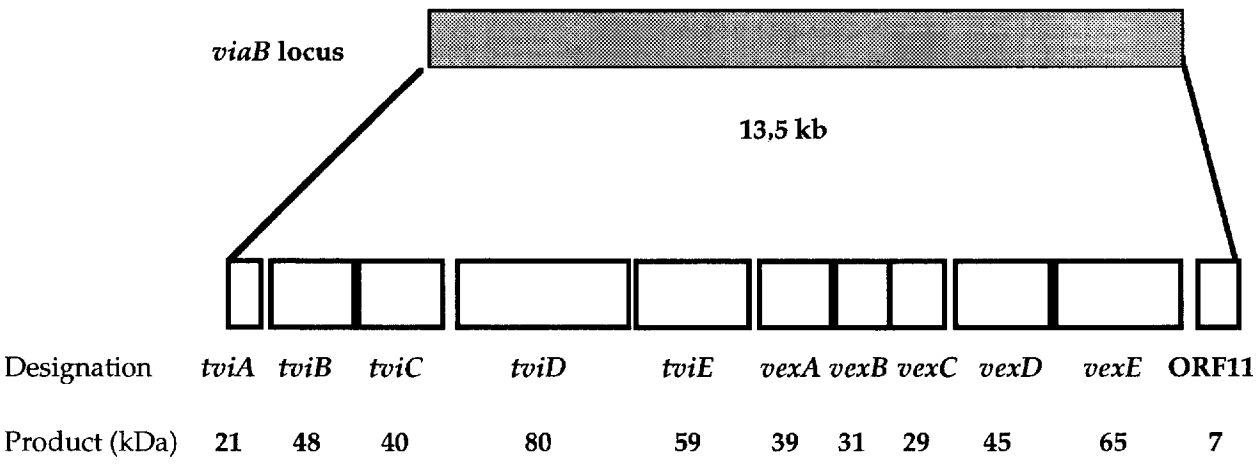

Fig. 2. Genetic organization of the *viaB* locus.

Position and designation of the open reading frames of the *viaB* locus (ORFs), and molecular mass (kDa) of the corresponding products.

charidic chains are then translocated from the cytoplasmic membrane through the periplasmic space and the outer membrane, and anchored to the cell surface to form the capsule. Lipid substitution is required at this step (Frosch and Müller, 1993).

The level of capsule expression is affected by regulators responding to environmental signals. Some of these regulators form the *rcs* system. This system was first identified in *E. coli* but homologs are known in other bacteria, such as *Klebsiella pneumoniae* (Allen *et al.*, 1987; McCallum and Whitfield, 1991), *Erwinia amylovora* (Coleman *et al.*, 1990) or *S. typhi* (Houng *et al.*, 1992). The regulatory strategies used for colanic acid biosynthesis, produced by *E. coli* K12, usually serve as the reference model for regulation of capsule synthesis (Stout, 1994).

The *rcs* system of *E. coli* K12 includes three positive regulators (RcsA, RcsB, and RcsF) and two negative regulators (RcsC and Lon). RcsB and RcsC act as the effector and the sensor, respectively, of a two-component regulatory system by stimulating capsule synthesis from colanic acid synthesis *cps* genes (Stout and Gottesman, 1990). The environmental signals, which activate RcsC, have yet to be determined. RcsA is an auxiliary factor which may interact with RcsB to form a heterodimer required for an increased transcription of *cps* genes and thus, for maximal synthesis of colanic acid. The availability of RcsA protein is normally limited because it is a substrate for the ATP-dependent Lon protease (Torres-Cabassa and Gottesman, 1987). In addition, Gervais and Drapeau (1992) proposed that RcsF could activate the effector RcsB through kinase activity, although phosphorylation of RcsB has not been directly demonstrated.

Biosynthesis of S. typhi *Vi antigen*

Knowledge of the Vi antigen biogenesis of *S. typhi* increased recently. Cloning of the *S. typhi viaB* locus provided an approach for studying the biogenesis of this antigen (Kolyva *et al.*, 1992; Hashimoto *et al.*, 1993). For this purpose, different mutagenesis strategies have been used. First, transposon Tn*5* mutagenesis of this locus showed that it contained at least two regions: one, comprising *tviB, tviC* and *tviE,* involved in the biosynthesis of Vi antigen and the other, including *vexA, vexB, vexC, vexD* and *vexE* genes, required for translocation of the polysaccharide (Hashimoto *et al.*, 1991; Kolyva *et al.*, 1992; Hashimoto *et al.*, 1993). More recently, our laboratory constructed *S. typhi* Ty2 strains carrying nonpolar mutations in 10 genes of the *viaB* locus to examine the individual contribution of each gene to the Vi phenotype. Phenotype of the mutants and primary amino acid sequence analysis of *viaB* determinants demonstrated that the *viaB* locus of *S. typhi* is involved not only in the synthesis and export process of Vi antigen, but also in the regulation of its expression (Virlogeux *et al.*, 1995).

Synthesis of Vi antigen requires functional TviB, TviC, TviD and TviE polypeptides (fig. 3). The corresponding *S. typhi* Ty2 mutants do not release any Vi polymer in culture supernatant, are devoid of capsular polysaccharide on the cell surface, and do not accumulate polymer in their intracellular compartment (Virlogeux *et al.*, 1995). Moreover, Vi polymer is detected in the intracellular compartment of *E. coli* HB101 only when the *tviB, tviC, tviD* and *tviE* genes, carried on plasmids, are introduced simultaneously in this bacteria (Hashimoto *et al.*, 1993; Virlogeux *et al.*, 1995). Considering the primary amino acid similarities of TviB and TviC with the GDP-mannose dehydrogenase of *Pseudomonas aeruginosa* and UDP-glucose 4-epimerases from prokaryotic and eukaryotic organisms, respectively, and the identification of a potential dinucleotide-binding site, Hashimoto *et al.* (1993) suggested that *tviB* and *tviC* might encode the NAD- or NADP-dependent enzymes required to synthesize the nucleotide sugar for Vi polysaccharide synthesis. Subsequent polymerization could be catalysed by the TviE protein. Indeed, the C-terminal domain of TviE presents a significant level of homology to UDP-galactosyltransferases of prokaryotic and eukaryotic organisms. In addition, synthesis of Vi antigen seems to require the TviD polypeptide, since a non-polar *tviD* mutant of *S. typhi* Ty2 fails to synthesize detectable Vi antigen (Virlogeux *et al.*, 1995). However, the function of this protein

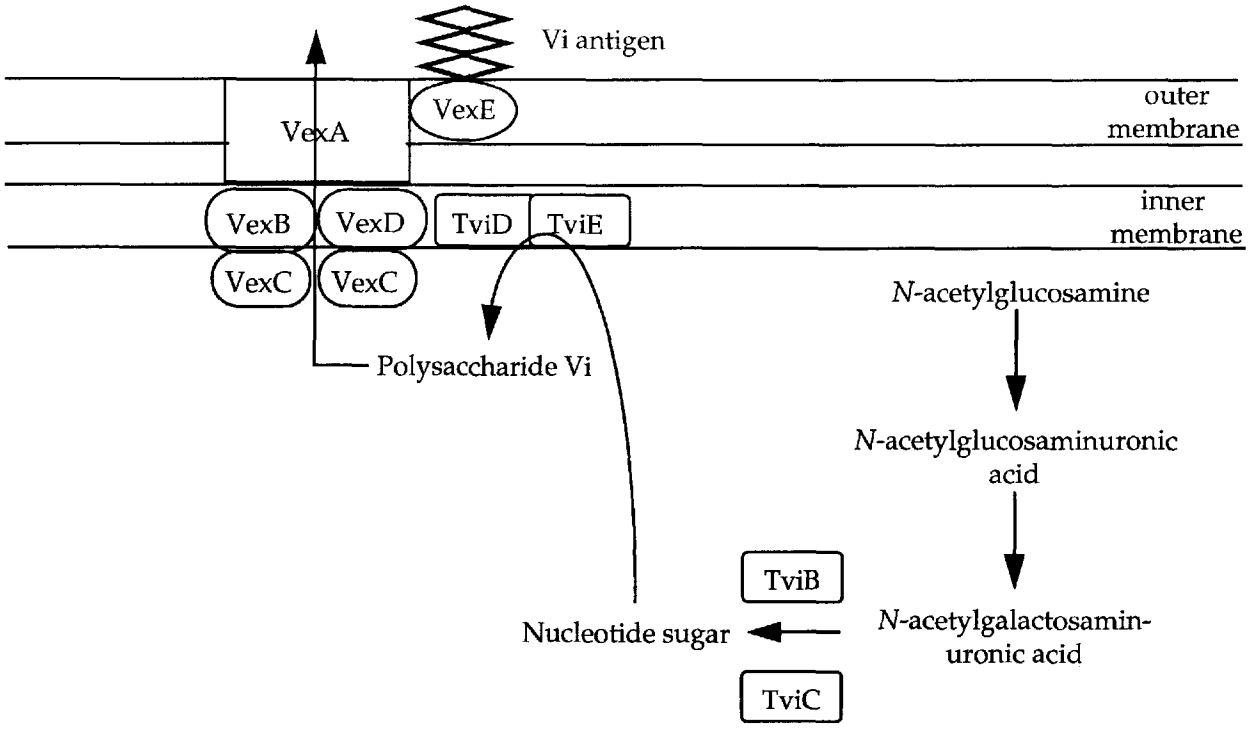

Fig. 3. Model for expression of the Vi antigen in *S. typhi.*

Monomers of *N*-acetylgalactosaminuronic acid are synthesized in the cytoplasmic compartment. TviB and TviC are the enzymes required for the activation of these monomers. Subsequent polymerization, at the endogenous lipid carrier TviD, is catalysed by TviE. Vi polysaccharide is then exported by the VexA, VexB, VexC and VexD proteins, which could form an ABC transporter. Finally, the Vi antigen is anchored to the cell surface by the VexE protein.

remains unclear. Two other different phenotypes were identified when the *tviD* gene was disrupted. A chromosomal transposon Tn*5* insertion in the *tviD* gene of *S. typhi* Ty2 caused the intracellular accumulation of Vi polymer (Kolyva *et al.*, 1992). In contrast, Vi polysaccharide was detected in the cytoplasmic compartment and on the cell surface of *E. coli* HB101 carrying multicopies of the *viaB* locus in which the *tviD* coding sequence had been disrupted by a transposon insertion and in which the transport system was functional (Kolyva *et al.*, 1992; Hashimoto *et al.*, 1993). This latest mutant was weakly agglutinated with an anti-Vi monoclonal antibody and the cell extract of this strain gave a faint and broad line of precipitation against an anti-Vi monoclonal antibody, suggesting that the antigen synthesized by this mutant probably had only partially antigenic properties of the Vi antigen (Hashimoto *et al.*, 1993). These observations suggest that TviE could initiate Vi chain elongation in the absence of TviD, but that these Vi chains are badly recognized by the components of the translocation system. From these data, we suggest that TviD protein might be the endogenous lipid carrier essential for the initiation of polymerization by TviE and for the polysaccharide translocation to the bacterial cell surface (fig. 3) (Virlogeux *et al.*, 1995).

Translocation of Vi antigen to the cell surface

Phenotype analysis of transposon or non-polar mutants demonstrates that at least four polypeptides encoded by the *viaB* locus are involved in

the Vi polysaccharide export apparatus (fig. 3) (Kolyva *et al.*, 1992; Hashimoto *et al.*, 1993; Virlogeux *et al.*, 1995). These polypeptides are the VexA, VexB, VexC and VexE proteins. No *vexD* mutant of *S. typhi* was obtained up to now. Mutants defective in VexA, VexB and VexC synthesis accumulate Vi polysaccharide in their cytoplasm, but no Vi polymer could be detected at the cell surface of the mutants or in the culture supernatant (Virlogeux *et al.*, 1995). In addition, VexA, VexB, VexC polypeptides and also VexD protein share primary amino acid similarities with components of *A*TP-*b*inding *c*assette transporters (ABC transporters) (Hashimoto *et al.*, 1993; Waxin *et al.*, 1993).

ABC transporters present the characteristic of utilizing energy from ATP hydrolysis to transport their substrate. Generally, they consist of two to four membrane-associated domains or polypeptides, one or two hydrophobic integral membrane proteins, and one or two hydrophilic ATP-binding proteins (Higgins, 1992; Fath and Kolter, 1993). In addition, some of these ATP-dependent export systems require auxiliary proteins to transport their substrate across the outer membrane (Fath and Kolter, 1993). These transport systems were previously identified only in bacteria expressing group II polysaccharide such as *N. meningitidis* (Frosch *et al.*, 1991), *H. influenzae* (Kroll *et al.*, 1990) and *E. coli* K1 (Silver, 1994). Proteins of the transport system of Vi antigen fit these characteristics. VexB is a highly hydrophobic protein with seven potential membrane-spanning domains and VexD has two hydrophobic segments of non-polar amino acids with a potential membrane-spanning domain (Hashimoto *et al.*, 1993). Thus, these two proteins, VexB and VexD, could be two integral membrane proteins of the Vi antigen export apparatus. In this transport system, VexC is probably the ATP-binding protein, as it is a relatively hydrophilic protein and it has the putative ATP-binding site GXGKT at amino acid position 56 following the GTG start codon (Hashimoto *et al.*, 1993).

In addition, translocation of Vi polysaccharide to the bacterial cell surface seems to require two other proteins: VexA and VexE (fig. 3). The VexA protein is similar to CtrA protein of *N. meningitidis*, which has a signal sequence and is localized in the outer membrane of this bacteria. Since VexA has a putative lipoprotein signal sequence with a possible cleavage site, Ala-22-Val-23, this protein may, like CtrA, also be localized in the outer membrane and could mediate translocation of the Vi polysaccharide across the outer membrane (Kolyva *et al.*, 1992; Hashimoto *et al.*, 1993; Waxin *et al.*, 1993).

Anchoring of the Vi antigen to the *S. typhi* cell surface is probably dependent on the VexE protein. Indeed, a *S. typhi* Ty2 *vexE* mutant was able to synthesize and export the Vi antigen but does not express it on its cell surface (Virlogeux *et al.*, 1995). VexE is the second protein necessary for the cell surface expression of capsule identified. Previously, Silver *et al.* (1987) showed that a chromosomal mutation in the *kpsD* gene of *E. coli* K1 resulted in loss of surface expression of the K1 capsule. KpsD and VexE share the characteristic of being two hydrophilic proteins with positively charged residues throughout the sequence, which may be relevant to interaction with the negatively charged polysaccharides. However, KpsD is a periplasmic protein, whereas VexE does not contain a characteristic signal sequence required for secretion to the periplasmic space. Moreover, no homology was detected between these two proteins (Virlogeux *et al.*, 1995).

In conclusion, the Vi antigen export apparatus is composed of five polypeptides: VexA, VexB, VexC and VexD, which could form an ABC transporter, and the VexE anchoring protein (fig. 3). The requirement of an ABC transporter for Vi antigen translocation suggests that a conserved mechanism may exist for the energy-dependent translocation of group I and group II capsular polysaccharides in Gram-negative bacteria.

Regulation of Vi antigen expression

The regulation of Vi antigen expression by *S. typhi* may have a significant role in maintaining appropriate levels of Vi production in the different host environments encountered by this bacterium. OmpR, RcsB and TviA proteins are

involved in this regulation. However, phenotypes of mutants defective in synthesis of these proteins are different. *ompR* and *rcsB* mutants of *S. typhi* are not able to synthesize Vi antigen, whereas a *S. typhi* Ty2 mutant defective in TviA synthesis produces Vi antigen, but a 20-fold less amount than that produced by the parental strain (Pickard *et al.*, 1994; Virlogeux *et al.*, 1995, 1996a).

Little is known about the regulation of Vi antigen expression by the *ompR-envZ* two component regulatory system. Pickard *et al.* (1994) have suggested that osmolarity could be an environmental signal involved in this regulation. When the NaCl concentration of the *S. typhi* growth medium rises 400 mM, Vi antigen expression by *S. typhi* Ty2 is markedly decreased. At 500 mM NaCl, this strain no longer expresses this antigen (Pickard *et al.*, 1994). The mechanism involved in this regulation remains to be determined.

Regulation of Vi antigen expression by RcsB and TviA is better known. By mRNA and gene fusion analyses, we have demonstrated that these two proteins are simultaneously required for cotranscription of *tviA-B* genes and thus, for Vi antigen synthesis (Virlogeux *et al.*, 1995, 1996a). In the absence of RcsB or TviA, transcription initiated at the *tviA* promoter terminates in the *tviA-tviB* intergenic region, probably at a putative hairpin structure identified in this region (Virlogeux *et al.*, 1996a). An interaction between these proteins was suggested by complementation experiments of two *rcsB* mutants of *S. typhi*. One of these mutants, T643WSR, is the original *viaA* mutant described by Johnson *et al.* (1965) and presents a punctual mutation in the potential DNA-binding motif of its RcsB product (Virlogeux *et al.*, 1996a). It was complemented either by introducing a recombinant plasmid carrying the *rcsB* gene or by a plasmid harbouring the *tviA* gene, a result inconsistent with the simultaneous requirement of RcsB and TviA for Vi antigen synthesis. However, the requirement of these two proteins was confirmed by complementation of the second *rcsB* mutant, Ty2*(rcsB)*, in which the *rcsB* gene was disrupted by a cassette insertion. This mutant could only be complemented by introducing a recombinant plasmid carrying the *rcsB* gene, suggesting that T643WSR was able to synthesize a RcsB* protein which could positively control Vi synthesis only when TviA was overexpressed. This assumption was confirmed by complementation of Ty2*(rcsB)* by a plasmid carrying the *rcsB** gene of T643WSR and another plasmid harbouring *tviA* (Virlogeux *et al.*, 1996a). Moreover, Hashimoto *et al.* (1996) demonstrated that TviA specifically bound to the 451-bp *Bsp*H1-*Xba*I fragment located upstream of the *tviA* gene. Analysis of the amino acid sequence of TviA revealed a putative helix-turn-helix DNA-binding motif in the C-terminal domain of this hydrophilic protein (Hashimoto *et al.*, 1996).

In conclusion, the regulation of Vi antigen synthesis depends on two two-component regulatory systems: the OmpR-EnvZ system and the RcsB-RcsC system (fig. 4). For the *rcs* system, we proposed a model in which the two positive regulators TviA and RcsB interact together to initiate the transcription of genes involved in the synthesis of Vi antigen in *S. typhi* (Virlogeux *et al.*, 1996a). Interestingly, it should be noted that, contrary to the mechanism involved in the regulation of colanic acid synthesis in *E. coli*, full expression of Vi antigen did not require the RcsA protein. An *rcsA* mutant of *S. typhi* Ty2 retains a Vi positive phenotype expressing Vi antigen as the parental strain (Virlogeux *et al.*, 1996a). These data constitute a major difference between regulation of Vi antigen expression in *S. typhi* and of colanic acid synthesis in *E. coli* by the *rcs* system.

Vi antigen as a potential virulence factor

Felix and Pitt (1934) called the surface antigen covering *S. typhi* "Vi antigen" for *vi*rulence based on its ability to enhance *S. typhi* virulence in mice. This observation was confirmed, among others, by Hone *et al.* (1988). These authors showed that the removal of Vi antigen from Ty2 was responsible for increasing the LD_{50} by a factor of 10^4 in the mouse mucin virulence assay compared with the LD_{50} of the parental strain Ty2 after intraperitoneal inoculation of the bacteria.

Epidemiological data also agreed with the importance of Vi antigen for the virulence of

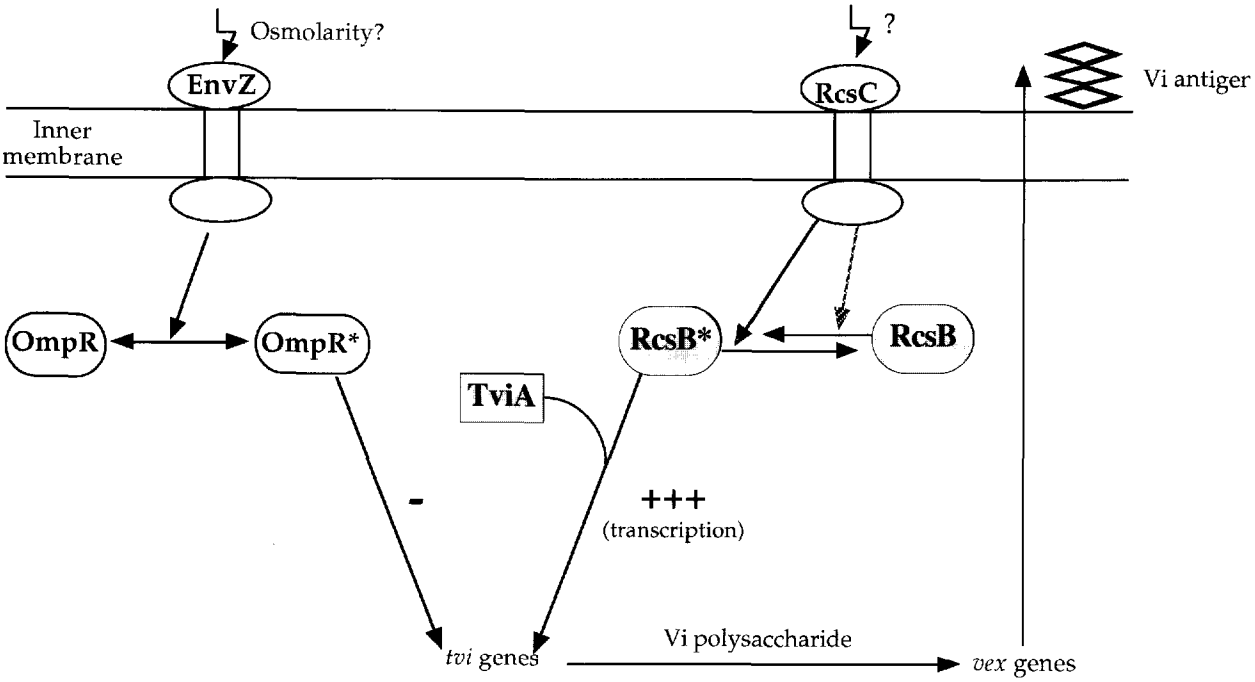

Fig. 4. Model of regulation of Vi antigen expression in *S. typhi.*

Two two-component regulatory systems are involved in the regulation of Vi antigen expression in *S. typhi.* The *rcs* system positively regulates transcription of *tvi* genes, whose products are required for Vi antigen synthesis. Moreover, interaction between TviA and RcsB proteins is necessary for maximal activation of *tvi* gene transcription. Environmental signals, which activate RcsC, have yet to be determined. OmpR/EnvZ is the second regulatory pair involved in the regulation of Vi antigen expression. An increase in the environmental osmolarity could lead to the negative regulation of Vi antigen synthesis by this system. Mechanisms involved in this regulation remain to be determined.

S. typhi for humans. All isolates of *S. typhi* from the blood of patients with typhoid fever invariably possess the Vi capsular polysaccharide (Craigie and Brandon, 1936). Similar results were obtained by Felix *et al.* (1935) who reported that 84 of 86 *S. typhi* isolates from blood possessed the Vi antigen. The two negative isolates were probably false-negative considering that they had been passaged several times before being tested and that the amount of Vi antigen decreased following multiple subcultures of *S. typhi* on agar.

The role of Vi antigen in the virulence of *S. typhi* is less convincing in clinical trials with volunteers. Hornick *et al.* (1970) tested the virulence of *S. typhi* strains expressing Vi antigen or not for healthy volunteers after the administration of 10^7 bacteria, which corresponds to the infectious dose provoking illness in 50% of the volunteers. In these experimental conditions, diseases rates were higher in volunteers who ingested Vi-containing strains than with non-Vi strains: 51 versus 26% (p less than 0.5). Vi antigen thus appeared to be an important determinant of human virulence for typhoid bacilli. However, these experiences also prove that a Vi-negative strain is able to induce typhoid fever in humans. Such a result was also obtained by Hone *et al.* (1988) with a *S. typhi* Ty2 (*galE,* Vi-negative) strain tested on volunteers. Two of the four volunteers developed an illness clinically indistinguishable from typhoid within 1 week of ingesting a single dose of 7×10^8 viable organisms, thus demonstrating that this Vi-negative strain retained virulence in humans.

Mechanisms of Vi antigen virulence were examined *in vitro.* Early workers found that the Vi antigen did not affect phagocytosis by PMNs

but that it increased the resistance against post-phagocytic oxidative burst (Kossack *et al.,* 1981; Miller *et al.,* 1972). However, methods utilized by these authors to measure the phagocytic rate were significantly different from those they used to measure oxidative burst. This information could explain the conflicting results obtained by Looney and Steigbigel (1986), who found a close, significant correlation between these two physiological steps. Indeed, they found that the Vi antigen decreased the fixation of C3 complement factor to the surface of *S. typhi* and that the Vi antigen-containing strains of *S. typhi* have decreased killing by serum, decreased killing via the alternative pathway of complement, and decreased opsonization as measured by phagocytosis by human PMNs. Thus, the decrease in oxidative burst by the Vi-containing strains of *S. typhi* was attributable by Looney and Steigbigel (1986) to the slower uptake of the organism by the PMNs, which, in turn, is a function of the decreased opsonization of these strains. These authors have also shown that strains of *S. typhi* containing the Vi antigen were less susceptible to killing by H_2O_2 than strains without this antigen. Recently, the role of Vi antigen in the resistance of the bactericidal effect of serum was confirmed with isogenic strains of *S. typhi* (Hashimoto *et al.,* 1993; Virlogeux *et al.,* 1996b). All these characteristics of Vi antigen are the main characteristics of capsules involved in the virulence of bacteria. However, the *in vivo* relevance of these mechanisms of resistance to host defence remains speculative.

In conclusion, available data show that *S. typhi* Vi antigen is not the main virulence factor of this bacteria, but that it probably takes part in the virulence of this *Salmonella* by interfering with certain non-specific defences of the host. However, the lack of a true experimental animal model is one of the main problems encountered for the demonstration of the relevance of this idea *in vivo.* The *S. typhi* mouse model used by many authors up to now is not the ideal one. Indeed, *S. typhi* is not an invasive pathogen for mice. Without gastric mucin or similar agents, which depress host resistance, infective doses above 10^7 bacteria are necessary to cause death in mice infected intraperitonally by *S. typhi* (Robbins and Robbins, 1984). Moreover, the experience of Hone *et al.* (1988) showed that *S. typhi* EX462 (*galE,* Vi-negatif) had very attenuated virulence for mice when compared with the parental strain Ty2H1*(galE),* but that this strain retained virulence in humans.

Vi antigen as a vaccine

Typhoid fever remains a public health problem in many developing areas of the world, causing more than 600,000 deaths annually (Ivanoff *et al.,* 1994). Despite effective treatment for typhoid fever (Mandal, 1991), some *S. typhi* strains resistant to several antibiotics have been reported (Rowe *et al.,* 1990, 1992; Mourad *et al.,* 1993). Development of vaccines which could be used as public health tools in developing countries is therefore necessary.

Capsular polysaccharides of pathogenic bacteria are generally immunogenic for humans and animals. The Vi antigen of *S. typhi* was first identified as an immunogenic capsular polysaccharide by Felix and Pitt (1934). These authors observed that the *S. typhi* strains, recently isolated from the blood of patients with typhoid fever, were agglutinated by sera of rabbits immunized with living *S. typhi.* On the other hand, they did not observe such agglutination when the rabbits were immunized with a bacterial suspension inactivated at 100°C. An immunological response was also observed by Gaines *et al.* (1966) in mice. Study of the immunochemical properties of the Vi antigen from *S. typhi* Ty2 showed the presence of at least two antigenic determinants. One of them is the O-acetyl moiety which plays a dominant role. The other antigenic determinant involves both carboxyl and *N*-acetyl groups (fig. 1) (Szewczyk and Taylor, 1980).

In humans, a poor response to Vi antigen is observed in acute typhoid fever which contrasts with the very high response in most chronic carriers (Felix *et al.,* 1935). From this observation of high anti-Vi antibodies response in healthy carriers emerged the idea of the protective role of these antibodies against typhoid fever. Since then, several studies were carried out to develop a vaccine constituted of Vi antigen. Initial studies using a purified form of Vi antigen as a vaccine

failed to protect either chimpanzees or humans (Landy, 1954). However, this failure was due to the denaturing technique used to prepared the Vi antigen and it was circumvented when a non-denaturing technique was used. In this latter case, the antigenic characteristics of Vi antigen were maintained and the parenteral Vi antigen vaccine was well tolerated and immunogenic (Robbins and Robbins, 1984; Tacket *et al.*, 1986). This vaccine elicited high anti-Vi antibody titres in about 90% of the recipients (Tacket *et al.*, 1986) and the anti-Vi antibodies generated in the vaccinees persisted for at least three years (Tacket *et al.*, 1988). The adverse reactions observed after vaccination include local pain and induration at the injection site in 10-20% of the vaccinees and, in a few rare cases, a low-grade fever (Archarya *et al.*, 1987; Hall, 1995).

Two field trials have been conducted to assess the safety and the efficacy of a single intramuscular dose of 25 μg of Vi antigen (Archarya *et al.*, 1987; Klugman *et al.*, 1987). In Nepal, the vaccine had a 72% protective rate for at least 17 months against culture-confirmed typhoid fever in subjects aged 5 to 44 years. Efficacy was correlated with the development of anti-Vi polysaccharide antibodies. Similar results were obtained in South Africa with schoolchildren aged 5 to 16 years, in whom 64% protection was observed for at least 21 months. A significant increase in anti-Vi antibodies was still seen, clearly establishing the efficacy of the Vi antigen vaccine based on humoral immunity to this antigen.

Purified Vi polysaccharide behaves like a T-lymphocyte-independent antigen (Robbins and Robbins, 1984). This property explains why the serum antibody response is not boosted by the administration of additional doses of Vi antigen vaccine and why the immunogenicity of Vi in infants is less than in older vaccinees. In an attempt to increase the immunogenicity of Vi as a parenteral vaccine, Szu *et al.* conjugated Vi antigen to carrier proteins, such as tetanus or diphtheria toxoids, cholera toxin, the B subunit of the heat-labile toxin of *E. coli* (LT-B) or the recombinant exoprotein A of *Pseudomonas aeruginosa* (rEPA) (Szu *et al.*, 1987, 1989, 1994). These candidate conjugate vaccines elicited higher levels of serum antibodies than purified Vi alone in mice and in rhesus monkey or guinea pigs. Subsequent inoculations of animals with Vi conjugate clearly boost the serum Vi antibody titre. Preliminary clinical characterization of Vi-LT-B and Vi-rEPA was realized in adult volunteers (Szu *et al.*, 1994). These conjugate vaccines elicited higher levels of antibodies than Vi alone after the first injection and 26 weeks later, but a second injection of the conjugates did not elicit a booster response of Vi antibodies. No side effects were observed in the vaccinees. Clinical studies of these Vi conjugate vaccines with adults and infants in areas where typhoid is endemic are underway. If these Vi conjugate vaccines are effective in endemic areas, advantages in utilizing them would include the elicitation of higher antibody titres following the initial dose of vaccine and the stimulation of a higher level of protection than the ≈65% efficacy stimulated by purified Vi vaccine. On the other hand, expected drawbacks include greater expense and the need for multiple doses to achieve maximal antibody titres and for a cold chain to maintain their stability. However, the observation of better immunogenicity of conjugate vaccines in young animals than with Vi alone supports the advantage of such vaccines. If these results are confirmed in young children and infants, Vi conjugate vaccines would be very good candidates for vaccination of these age groups in areas where typhoid is endemic.

Conclusion

In this review, we have seen that *S. typhi* Vi antigen exhibits two major attributes: as a virulence factor of *S. typhi* and as a vaccine against typhoid fever. The role of the Vi antigen in the virulence of *S. typhi* remains a much debated question because of the lack of an animal experimental model. However, new approaches for studying the virulence of *S. typhi* are now being considered following the cloning and characterization of the *viaB* locus. One of these approaches consists of the study of the regulation of Vi antigen expression. Indeed, the finding that this expression in *S. typhi* is regulated by members of the family of two-component systems, which are known to be

important in controlling gene expression *in vivo*, suggests that there is a need to regulate the expression of Vi antigen in different host microenvironment in order for *S. typhi* to express full virulence. The only environmental signal known today is osmolarity, and *S. typhi* encountered environments in which osmolarity differed during the stages of pathogenesis. In the intestinal lumen, osmolarity is high (≥0.3 M NaCl). At this osmolarity, the invasiveness of *S. typhi* into tissue culture cells is maximal and the bacteria seems not to express Vi antigen (Tartera and Metcalf, 1993). On the other hand, in the bloodstream, *S. typhi* encounters an osmolarity equivalent to 150 mM NaCl (Miller and Mekalanos, 1988) and expresses the Vi antigen, which is thought to be important for resistance to serum activity. Thus, the identification of other environmental signals involved in regulation of Vi antigen expression would be advantageous in the formulation of new hypotheses for the role of Vi antigen in virulence.

To offset the lack of an animal model, one approach could consist of the construction of recombinant strains of different *Salmonella* serotypes expressing the Vi antigen of *S. typhi* for testing in animals in which these *Salmonella* are natural invasive pathogens. We have begun this work by the construction of recombinant strains of *S. typhimurium* expressing or not the Vi antigen of *S. typhi,* since *S. typhimurium* is able to induce a typhoid-like syndrome in mice. However, these strains were as virulent as the parental strains in the mouse virulence assay (Virlogeux *et al.*, 1996b). Another *Salmonella* serotype of interest is *S. dublin.* A few strains of *S. dublin* express Vi antigen, but this expression is not stable (Le Minor and Nicolle, 1964). Construction of a *S. dublin* recombinant strain stably expressing the Vi antigen of *S. typhi* would be of interest for testing the role of this antigen in virulence.

Immunogenic and protective properties of Vi antigen have been used for the development of vaccines against typhoid fever. The purified Vi polysaccharide vaccine is henceforth a good candidate for use in public health control programmes. It is currently licensed by the Merieux Institute ("Typhim Vi") in Chile, the Congo, Ivory Coast, France, the Republic of Korea, the Netherlands, Peru, the Philippines, Togo, the United Kingdom (Ivanoff *et al.*, 1994) and recently in the United States (Hall, 1995). However, further evaluation is needed for safety and protective efficacy of this vaccine in school-age children who account for the highest incidence of typhoid fever and are amenable to the school-based immunization programme. Such studies are in progress.

Acknowledgements

We thank P. Pardon and P. Velge for critical review of this manuscript.

L'antigène Vi de *Salmonella typhi*

Salmonella typhi, l'agent étiologique de la fièvre typhoïde humaine, exprime un polyoside capsulaire appelé antigène Vi. Certains auteurs ont impliqué cet antigène dans la virulence de *S. typhi,* plus particulièrement dans la résistance à l'activité bactéricide du sérum humain normal. Cependant, *S. typhi* étant une bactérie strictement adaptée à l'homme, le rôle de l'antigène Vi dans la virulence de cette bactérie reste controversé faute de modèle expérimental animal. Récemment, pour une meilleure appréhension du rôle de cet antigène dans la virulence, l'étude des mécanismes impliqués dans la biogenèse de l'antigène Vi a été entreprise. Il apparaît que l'expression de cet antigène est régulée par au moins deux systèmes de régulation à deux composants, les systèmes OmpR-EnvZ et RcsB-RcsC, ce qui suggère un rôle important du micro-environnement rencontré par *S. typhi* dans l'expression de l'antigène Vi, et par là même, de sa virulence.

Par ailleurs, les propriétés immunogéniques et protectrices de l'antigène Vi ont été utilisées à des fins vaccinales. Un vaccin polyosidique, constitué exclusivement d'antigène Vi, a été mis au point. La protection observée dans des régions où la fièvre typhoïde est endémique, est supérieure à 65 % et, trois ans après la vaccination, les titres d'anticorps anti-Vi restent très élevés. Le développement de vaccins constitués d'antigène Vi conjugués à des protéines porteuses est en cours, dans le but, notamment, d'obtenir l'augmentation des taux de protection contre la fièvre typhoïde chez les enfants.

Mots-clés: Salmonella typhi, Antigène Vi; Virulence, Vaccin, Biogenèse; Revue.

References

Acharya, I.L., Lowe, C.U., Thapa, R., Gurubacharya, V.L., Shrestha, M.B., Cadoz, M., Schulz, D., Armand, J., Bryla, D.A., Trollfors, B., Cramton, T., Schneerson, R. & Robbins, J.B. (1987), Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of *Salmonella typhi. N. Engl. J. Med.,* 317, 1101-1104.

Allen, P., Hart, C.A. & Saunders, J.R. (1987), Isolation from *Klebsiella* and characterization of two *rcs* genes that activate colanic acid capsular biosynthesis in *Escherichia coli. J. Gen. Microbiol.,* 133, 331-340.

Baker, E.E., Whiteside, R.E., Basch, R. & Derow, M.A. (1959), The Vi antigen of the *Enterobacteriaceae.* — II. Immunologic and biologic properties. *J. Immunol.,* 83, 680-686.

Boulnois, G.J. & Roberts, I.S. (1990), Genetics of capsular polysaccharide production in bacteria, *in* "Current Topics in Microbiology and Immunology" (K. Jann & B. Jann), 150, pp. 1-18, Springer Verlag, Berlin.

Coleman, M., Pearce, R., Hitchin, E., Busfield, F., Mansfield, J.W. & Roberts, I.S. (1990), Molecular cloning, expression and nucleotide sequence of *rcsA* gene of *Erwinia amylovora,* encoding a positive regulator of capsule expression: evidence for a family of related capsule activator proteins. *J. Gen. Microbiol.,* 136, 1799-1806.

Craigie, J. & Brandon, K.F. (1936), The identification of the V and W forms of *B. typhosus* and the occurrence of the V form in cases of typhoid fever and in carriers. *J. Pathol. Bacteriol.,* 43, 249-260.

Daniels, E.M., Schneerson, R., Egan, W.M., Szu, S.C. & Robbins, J.B. (1989), Characterization of the *Salmonella paratyphi* C Vi polysaccharide. *Infect. Immun.,* 57, 3159-3164.

Fath, M.J. & Kolter, R. (1993), ABC transporters: bacterial exporters. *Microbiol. Rev.,* 57, 995-1017.

Felix, A. & Pitt, R.M. (1934), A new antigen of *B. typhosus. Lancet,* 227, 186-191.

Felix, A., Krikorian, K.S. & Reitler, R. (1935), The occurrence of typhoid bacilli containing Vi antigens in cases of typhoid fever and of Vi antibody in their sera. *J. Hyg.,* 35, 421-427.

Felix, A. & Pitt, R.M. (1936), The Vi antigens of various *Salmonella* types. *Brit. J. Exp. Pathol.,* 17, 81-86.

Frosch, M., Edwards, U., Bousset, K., Krauβe, B. & Weisgerber, C. (1991), Evidence for a common molecular origin of the capsule gene loci in Gram-negative bacteria expressing group II capsular polysaccharides. *Mol. Microbiol.,* 5, 1251-1263.

Frosch, M. & Müller, A. (1993), Phospholipid substitution of capsular polysaccharides and mechanisms of capsule formation in *Neisseria meningitidis. Mol. Microbiol.,* 8, 483-493.

Gaines, S., Currie, J.A. & Tully, J.G. (1966), Induction of immunological unresponsiveness to Vi antigen. *Ann. Inst. Pasteur,* 110, 60-68.

Gervais, F.G. & Drapeau, G.R. (1992), Identification, cloning, and characterization of *rcsF,* a new regulator gene for exopolysaccharide synthesis that suppresses the division mutation *ftsZ84* in *Escherichia coli* K12. *J. Bacteriol.,* 174, 8016-8022.

Gottesman, S. & Stout, V. (1991), Regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12. *Mol. Microbiol.,* 5, 1599-1606.

Hall, C. (1995), A single shot at *Salmonella typhi:* a new typhoid vaccine with pediatric advantages. *Pediatrics,* 96, 348-350.

Hashimoto, Y., Ezaki, T., Li, N. & Yamamoto, H. (1991), Molecular cloning of the *ViaB* region of *Salmonella typhi. FEMS Microbiol. Lett.,* 90, 53-56.

Hashimoto, Y., Li, N., Yokoyama, H. & Ezaki, T. (1993), Complete nucleotide sequence and molecular characterization of ViaB region encoding Vi antigen in *Salmonella typhi. J. Bacteriol.,* 175, 4456-4465.

Hashimoto, Y., Quayum Khan, A. & Ezaki, T. (1996), Positive autoregulation of *vipR* expression in ViaB region-encoded Vi antigen of *Salmonella typhi. J. Bacteriol.,* 178, 1430-1436.

Heyns, K. & Kiessling, G. (1967), Strukturaufklarung des Vi-antigens aus *Citrobacter freundii (E. coli)* 5396/38. *Carbohydr. Res.,* 3, 340-352.

Higgins, C. (1992), ABC transporters: from microorganisms to man. *Annu. Rev. Cell Biol.,* 8, 67-113.

Hone, D.M., Attridge, S.R., Forrest, B., Morona, R., Daniels, D., La Brooy, J.T., Chiron, R., Bartholomeusz, A., Shearman, D.J.C. & Hackett, J. (1988), A *galE via* (Vi antigen-negative) mutant of *Salmonella typhi* Ty2 retains virulence in humans. *Infect. Immun.,* 56, 1326-1333.

Hornick, R.B., Greisman, S.E., Wooward, T.E., Dupont, H.L., Dawkins, A.T. & Snyder, M.J. (1970), Typhoid fever: pathogenesis and immunologic control. *N. Engl. J. Med.,* 293, 686-691.

Houng, H.H., Noon, K.F., Ou, J.T. & Baron, L.S. (1992), Expression of Vi antigen in *Escherichia coli* K-12: characterization of ViaB from *Citrobacter freundii* and identity of ViaA with RcsB. *J. Bacteriol.,* 174, 5910-5915.

Ivanoff, B., Levine, M.M. & Lambert, P.H. (1994), Vaccination against typhoid fever: present status. *Bull. WHO,* 72, 957-971.

Jann, B. & Jann, K. (1990), Structure and biosynthesis of the capsular antigens of *Escherichia coli, in* "Current Topics in Microbiology and Immunology" (K. Jann & B. Jann) (pp. 19-42). Springer Verlag, Berlin.

Jennings, H.J. (1983), Capsular polysaccharides as human vaccines. *Adv. Carbohydr. Chem. Biochem.,* 41, 155-208.

Jennings, H.J. (1990), Capsular polysaccharides as vaccine candidates, *in* "Current Topics in Microbiology and Immunology" (K. Jann & B. Jann) (pp. 97-127). Springer Verlag, Berlin.

Johnson, E.M., Krauskopf, B. & Baron, L.S. (1965), Genetic mapping of Vi and somatic antigenic determinants in *Salmonella. J. Bacteriol.,* 90, 302-308.

Johnson, E.M. & Baron, L.S. (1969), Genetic transfer of the Vi antigen from *Salmonella typhosa* to *Escherichia coli. J. Bacteriol.,* 99, 355-359.

Klugman, K.P., Gilbertson, I.T., Koornshof, H.J., Robbins, J.B., Schneerson, R., Schulz, D., Cadoz, M. & Armand, J. (1987), Protective activity of Vi capsular polysaccharide vaccine against typhoid fever. *Lancet,* 2, 1165-1169.

Kolyva, S., Waxin, H. & Popoff, M.Y. (1992), The Vi antigen of *Salmonella typhi:* molecular analysis of the *viaB* locus. *J. Gen. Microbiol.,* 138, 297-304.

Kossack, R.E., Guerrant, R.L., Densen, P., Schadelin, J. & Mandell, G.L. (1981), Diminished neutrophil oxida-

tive metabolism after phagocytosis of virulent *Salmonella typhi. Infect. Immun.*, 31, 674-678.

Kroll, J.S., Loynds, B., Brophy, N. & Moxon, E.R. (1990), The *vex* locus in encapsulated *Haemophilus influenzae:* a chromosomal region involved in capsule polysaccharide export. *Mol. Microbiol.*, 4, 1853-1862.

Landy, M. (1954), Studies on Vi antigens: immunization of human beings with purified Vi antigen. *Am. J. Hyg.*, 60, 52-62.

Le Minor, L. & Nicolle, P. (1964), Sur deux souches de *Salmonella dublin* possédant l'antigène Vi. *Ann. Inst. Pasteur,* 107, 550-556.

Looney, R.J. & Steigbigel, R.T. (1986), Role of the Vi antigen of *Salmonella typhi* in resistance to host defense *in vitro. J. Lab. Clin. Med.*, 108, 506-516.

Mandal, B.K. (1991), Modern treatment of typhoid fever. *J. Infect. Dis.*, 22, 1-4.

Masson, L. & Holbein, B.E. (1983), Physiology of sialic acid capsular polysaccharide synthesis in serogroup B *Neisseria meningitidis. J. Bacteriol.*, 154, 728-736.

McCallum, K.L. & Whitfield, C. (1991), The *rcsA* gene of *Klebsiella pneumoniae* O1:K20 is involved in expression of the serotype-specific K (capsular) antigen. *Infect. Immun.*, 59. 494-502.

Miller, R.M., Garbus, J. & Hornick, R.B. (1972), Lack of enhanced oxygen consumption by polymorphonuclear leukocytes on phatocytosis of virulent *Salmonella typhi. Science,* 175, 1010-1011.

Miller, V.L. & Mekalanos, J.J. (1988), A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR. J. Bacteriol.*, 170, 2575-2583.

Mourad, A.S., Metwally, M. & Deen, A.N.E. (1993), Multidrug-resistant *Salmonella typhi. Clin. Infect. Dis.*, 17, 135-136.

Pickard, D., Li, J., Roberts, M., Maskell, D., Hone, D., Levine, M., Dougan, G. & Chatfield, S. (1994), Characterization of defined *ompR* mutants of *Salmonella typhi: ompR* is involved in the regulation of Vi polysaccharide expression. *Infect. Immun.*, 62, 3984-3993.

Puente, J.L., Verdugo-Rodriguez, A. & Calva, E. (1991), Expression of *Salmonella typhi* and *Escherichia coli* OmpC is influenced differently by medium osmolarity: dependence on *Escherichia coli* OmpR. *Mol. Microbiol.*, 5, 1205-1210.

Robbins, J.B. (1978), Vaccines for the prevention of encapsulated bacterial diseases: current status, problems and prospects for the future. *Immunochemistry,* 15, 839-854.

Robbins, J.D. & Robbins, J.B. (1984), Reexamination of the protective role of the capsular polysaccharide (Vi antigen) of *Salmonella typhi. J. Infect. Dis.*, 150, 436-449.

Robbins, J.B., Schneerson, R. & Szu, S.C. (1995), Perspective: Hypothesis: Serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. *J. Infect. Dis.*, 171, 1387-1398.

Rowe, B., Ward, L.R. & Threlfall, E.J. (1990), Spread of multi-resistant *Salmonella typhi. Lancet,* 336, 1065-1066.

Rowe, B., Ward, L.R. & Threlfall, E.J. (1992), Ciprofloxacin and typhoid fever. *Lancet,* 339, 740.

Sanderson, K.E. & Hurley, J.A. (1987), Linkage map of *Salmonella typhimurium, in "Escherichia coli* and *Salmonella typhimurium"* (F.C. Neidhardt, J.L. Ingraham, K. Brooks Low, M.B. Magasanik, M. Schaechter, M. & H.E. Umbarger) (pp. 877-918). American Society for Microbiology, Washington.

Silver, R.P., Aaronson, W. & Vann, W.F. (1987), Translocation of capsular polysaccharides in pathogenic strains of *Escherichia coli* requires a 60-kilodalton periplasmic protein. *J. Bacteriol.*, 169, 5489-5495.

Silver, R.P. (1994), Genetic analysis of the *Escherichia coli* K1 capsule gene cluster, *in* "Molecular genetics of bacterial pathogenesis" (V.L. Miller, J.B. Kaper, D.A. Portnoy & R.R. Isberg) (pp. 313-323). American Society for Microbiology, Washington.

Stone, A.L. & Szu, S.C. (1988), Application of optical properties of the Vi capsular polysaccharide for quantitation of the Vi antigen in vaccines for typhoid fever. *J. Clin. Microbiol.*, 26, 719-725.

Stout, V. & Gottesman, S. (1990), RcsB and RcsC: a two-component regulator of capsule synthesis in *Escherichia coli. J. Bacteriol.*, 172, 659-669.

Stout, V. (1994), Regulation of capsule synthesis includes interactions of the RcsC/RcsB regulatory pair. *Res. Microbiol.*, 145, 389-392.

Szewczyk, B. & Taylor, A. (1980), Immunochemical properties of Vi antigen from *Salmonella typhi* Ty2: presence of two antigenic determinants. *Infect. Immun.*, 29, 539-544.

Szu, S.C., Stone, A.L., Robbins, J.D., Schneerson, R. & Robbins, J.B. (1987), Vi capsular polysaccharide-protein conjugates for prevention of typhoid fever. Preparation, characterization, and immunogenicity in laboratory animals. *J. Exp. Med.*, 166, 1510-1524.

Szu, S.C., Schneerson, R., Vickers, J.H., Bryla, D. & Robbins, J.B. (1989), Comparative immunogenicities of Vi polysaccharide-protein conjugates composed of cholera toxin or its B subunit as a carrier bound to high- or low-molecular weight-Vi. *Infect. Immun.*, 57, 3823-3827.

Szu, S.C., Taylor, D.N., Trofa, A.C., Clements, J.D., Shiloach, J., Sadoff, J.C., Bryla, D. & Robbins, J.B. (1994), Laboratory and preliminary clinical characterization of Vi capsular polysaccharide-protein conjugate vaccines. *Infect. Immun.*, 62, 4440-4444.

Tacket, C.O., Ferreccio, C., Robbins, J.B., Tsai, C.M., Schulz, D., Cados, M., Goodeau, A. & Levine, M.M. (1986), Safety and immunogenicity of two *Salmonella typhi* Vi capsular polysaccharide vaccines. *J. Infect. Dis.*, 154, 323-345.

Tacket, C.O., Levine, M.M. & Robbins, J.B. (1988), Persistence of antibody titres three years after vaccination with Vi polysaccharide vaccine against typhoid fever. *Vaccine,* 6, 307-308.

Tartera, C. & Metcalf, E.S. (1993), Osmolarity and growth phase overlap in regulation of *Salmonella typhi* adherence to and invasion of human intestinal cells. *Infect. Immun.*, 61, 3084-3089.

Torres-Cabassa, A.S. & Gottesman, S. (1987), Capsule synthesis in *Escherichia coli* K12 is regulated by proteolyse. *J. Bacteriol.*, 169, 981-989.

Troy, F.A. & McCloskey, M.A. (1979), Role of a membranous sialyltransferase complex in the synthesis of surface polymers containing polysialic acid in *Esche-*

richia coli. Temperature-induced alteration in the assembly process. *J. Biol. Chem.*, 254, 7377-7387.

Tsang, R.S.W. & Wong, A. (1989), Release of Vi antigens from *Salmonella typhi:* implications for virulence and diagnosis. *FEMS Microbiol. Immunol.*, 47, 437-442.

Vimr, E.R. & Steenbergen, S.M. (1993), Mechanisms of polysialic acid assembly in *Escherichia coli* K1: a paradigm for microbes and mammals, *in* "Polysialic acid" (J. Roth, U. Rutishauser & F.A. Troy II) (pp. 73-91). Birkhäuser Verlag, Basel.

Virlogeux, I., Waxin, H., Ecobichon, C. & Popoff, M.Y. (1995), Role of the *viaB* locus in synthesis, transport and expression of *Salmonella typhi* Vi antigen. *Microbiology,* 141, 3039-3047.

Virlogeux, I., Waxin, H., Ecobichon, C., Lee, J.O. & Popoff, M.Y. (1996a), Characterization of the *rcsA* and *rcsB* genes from *Salmonella typhi: rcsB* through *tviA* is involved in regulation of Vi antigen synthesis. *J. Bacteriol.*, 178, 1691-1698.

Virlogeux, I., Waxin, H., Ecobichon, C., Hermant, D. & Popoff, M.Y. (1996b), Contribution de l'antigène Vi à la virulence de *Salmonella typhi. Méd. Mal. Infect. (in press).*

Waxin, H., Virlogeux, I., Kolyva, S. & Popoff, M.Y. (1993), Identification of six open reading frames in the *Salmonella enterica* subsp. *enterica* ser. Typhi *viaB* locus involved in Vi antigen production. *Res. Microbiol.*, 144, 363-371.