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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 encapsulated 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).

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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 virulence, 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 C-3 position.

\[ \text{NAc} = \text{N-acetyl group}; \text{AcO} = \text{O-acetyl group}; \bullet = \text{hydrogen.} \]

ORF = open reading frame.
This locus is located on a 13.5-kb chromosomal DNA fragment and contains 11 open reading frames (ORF), designated \( tviA-E \) (typhi Vi), \( vexA-E \) (Vi antigen export) 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.

### 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 progressively 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-

### Table: Designation and product (kDa)

| Designation | \( tviA \) | \( tviB \) | \( tviC \) | \( tviD \) | \( tviE \) | \( vexA \) | \( vexB \) | \( vexC \) | \( vexD \) | \( vexE \) | ORF11 |
|-------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-------|
| Product (kDa) | 21      | 48      | 40      | 80      | 59      | 39      | 31      | 29      | 45      | 65      | 7     |

**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 Tn5 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 vexF 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 non-polar 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
remains unclear. Two other different phenotypes were identified when the tviD gene was disrupted. A chromosomal transposon Tn5 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 ATP-binding cassette 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 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 *BspH1-XbaI* 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 virulence 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 LD50 by a factor of 10^4 in the mouse mucin virulence assay compared with the LD50 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
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 10⁷ 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 7×10⁸ 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 to 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 H2O2 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 10⁷ bacteria are necessary to cause death in mice infected intraperitoneally 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 prepare 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 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 Escherichia 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.

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L’antigène Vi de Salmonella typhi

Salmonella typhi, l’agent étiologique de la fièvre typhoïde humaine, exprime un polysaccharide 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 compréhension du rôle de cet antigène dans la virulence, l’étude des mécanismes impliqués dans la biogénèse 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 ResB-ResC, 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, Biogénèse; Revue.
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