The Roles of IS630 Sequence in the Expression of the Form I Antigen of *Shigella sonnei*: Molecular and Evolutionary Aspects

Huo-Shu H. Houng*, Michael J. Zapor, Antoinette B. Hartman, Thomas L. Hale and Malabi M. Venkatesan

Infections by *Shigella* spp. are frequent causes of human diarrheal diseases. These organisms invade the colonic epithelium via the distinct processes of penetration, multiplication and intercellular dissemination. One of the four species in this genus, *S. sonnei* comprises a single serotype expressing an O-chain lipopolysaccharide (LPS) termed form I (smooth) antigen. Unlike the other enteric organisms, the form I antigen of *S. sonnei* is encoded by a 180 kilobase (kb) virulence plasmid which is also essential for *Shigella* to invade epithelial cells. The expression of the form I antigen in *S. sonnei* is not stably maintained, frequently giving rise to a form II (rough) phenotype when cultivated on artifical medium. The form II colonies are cured of the invasive plasmid and are uniformly avirulent.

We have genetically characterized the form I coding region of *S. sonnei* and have found that an 11.0 kb *Hin*dIII-*Xba*I fragment is sufficient to encode for the form I antigen as detected by slide agglutination using form I-specific antisera. Precise boundaries for the form I coding region were defined by *Exo*III exonuclease mapping. A total of 10 essential ORF's organized as an operon were predicted from the DNA sequence analysis of the form I coding region. These polypeptides share significant homology with the products encoded by the Vi antigen genes of *S. typhi* and the *rfb* loci of *S. typhimurium* and *V. cholerae.* We also identified an insertional element sequence, IS630, which encodes for the 5th ORF of the form I operon in *S. sonnei.* Southern hybridization experiments have shown that *Plesiomonas shigelloides* and *S. sonnei* share homologous form I coding sequences. Using polymerase chain reactions (PCR) and available oligonucleotide primers flanking the IS630 sequence, we demonstrated that the form I coding region of *P. shigelloides* shares extensive homologous DNA sequence with *S. sonnei* form I antigen, but lacks the IS630 sequence. This may account for the comparative stability of the form I antigen expression in *P. shigelloides* over *S. sonnei.* Clinical isolates of *S. sonnei* from south America, southeast Asia, U.S.S.R., and United States were obtained and examined for the existence of IS630 in the form I coding region, and it was found that all the strains examined contain an IS630 sequence locating at the same insertion site within the from I coding region. We further constructed a recombinant plasmid encoding for the functional form I region which is free of IS630 sequence. The

resultant plasmid, pHH2062, is capable of expressing the form I antigen in *E. coli* K12, but fails to express the same form I antigen in its parental *S. sonnei* form II strain.

The form I coding regions of *S. sonnei* and *P. shigelloides* produce seriologically-identical O-antigen and have extensive homologous identities at DNA level. It is likely that the O-antigen operons for these two organisms evolved from the same origin. Results of this study indicated that the IS630 does not play any synthetic role in the form I antigen expression, but it plays an important role in terms of maintaining the form I expression and subsequent survival for the pathogenic *S. sonnei* in the human reservoir.

Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, DC 20307-5100