Construction of *Salmonella* vaccines

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Abstract

In this thesis the molecular cloning of the *gal* operon of *Salmonella typhimurium* LT2, the localisation of the *gal* promoter, and the *gal* genes: *galE, galT*, and *galK*, are described. The order of the genes and the direction of transcription was the same as occurs in *E. coli* K12 (promoter, *galE, galT, galK*). A restriction enzyme map of the operon was obtained and the termini of the three genes were precisely located by means of transposon insertion mutagenesis and minicell analysis of the *gal* proteins expressed by various plasmids.

A plasmid which contained a defined 0.4 kilobase deletion in the *galE* gene, but which still expressed *galT* and *galK* activities from the *gal* promoter was constructed. The *galE* deletion was recombined into the chromosomal *gal* operons of *S. typhimurium* and *Salmonella typhi* Ty2. The resulting strains were vigorous, non-reverting *galE* mutants which were sensitive to galactose–induced lysis at 0.06mM galactose. The *S. typhimurium galE* derivatives were avirulent and protective in mice whereas a *galE* mutant of *S. typhi* Ty2 (Ty2H1) was only partially attenuated in mice.

A rifampicin-resistant, Vi-negative (*via*) derivative of Ty2H1, strain EX462, was selected. Compared with the Ty2 parent strain, EX462 was serum–sensitive and highly attenuated in the mouse mucin virulence assay. When four human volunteers ingested 7×10^8 viable EX462, two became ill and developed a typhoid-like disease with fever and bacteremia. Blood isolates from these individuals were indistinguishable from the vaccine strain using a variety of criteria. We concluded that, even in a *via* background, the *galE* mutation was not attenuating for *S. typhi* in man.

A system was developed, whereby heterologous DNA encoding a protective antigen from an enteropathogen may be recombined into the chromosome of attenuated *Salmonella* carrier strains. This system involved: i) integration of a *hisOG* deletion mutation into the chromosome; ii) replacement of the *hisOG* deletion by the complete *hisOG* region and the segment of heterologous DNA

which encodes the antigen of interest. Recombinants were selected *his*$^+$. This system was used to integrate the pilin–encoding K88 genes of porcine enterotoxigenic *Escherichia coli* into the chromosome of a *galE* mutant of *S. typhimurium* (LT2H1). Recombinants were detected at a frequency of between 1.0×10^{-3} and 1.5×10^{-3}. A variety of tests confirmed that the K88 genes were integrated into the chromosome of LT2H1 and were expressed. The stability of the recombinant was tested both *in vivo* and *in vitro*. When administered orally to mice, the recombinant elicited a serum antibody response to K88, and retained the vaccine potential of the vector strain.