Functional Characterisation of the SefA Protein of *Salmonella enterica* serovar Enteritidis

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ABSTRACT

This study was commenced as a continuation of earlier work which used sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)-fractionated proteins to define the antigens of an attenuated *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) strain, *S.* Enteritidis 11RX (11RX) able to stimulate *in vitro* proliferation of, and cytokine release from T cells harvested from 11RX-primed (C57BL/6 × BALB/c) F1 mice. A highly immunogenic 14 kDa protein was identified, it was purified to homogeneity and was free from LPS contamination. The protein was able to elicit delayed-type hypersensitivity reactions in 11RX-primed mice, and stimulated *in vitro* proliferation of, and cytokine release from T cells obtained from these animals. The protein is the mature form of the 16 kDa precursor polypeptide previously described, and was established to correspond to the structural subunit protein (SefA) of *S.* Enteritidis fimbriae (SEF14) described by other workers.

The role of SefA in the biology of *S.* Enteritidis was investigated. This necessitated cloning and sequencing the *sefA* gene which was accomplished using a cosmid library of the 11RX chromosome. To define the role of SefA in the pathogenesis of *S.* Enteritidis, *sefA* was mutated in the chromosome of strain 11RX and in a virulent *S.* Enteritidis strain 7314 by allelic exchange with a copy that has been inactivated by insertion of a nonpolar kanamycin resistance (*aphA-3*) cassette. The effect of this mutation on the abilities of the mutated *S.* Enteritidis strains to colonise the Peyer's patches and to invade the spleen was assessed in BALB/c mice, and *in vitro* by adherence and invasion of HeLa cells. The results obtained showed that 11RX colonised better and persisted longer in the Peyer's patches, but not in the spleens, of these mice when compared to its SefA-deficient counterpart. In contrast, no such difference was observed between the virulent strain 7314 and its SefA-deficient counterpart. These findings were

correlated with *in vitro* adherence and invasion of HeLa cells. Furthermore, there was no demonstrable role for SefA in the virulence of *S.* Enteritidis as assessed by LD_{50} measurements in BALB/c mice.

The role of SefA in protection of mice against challenge by the virulent strain 7314 was investigated. Strong, specific antibody responses were mounted against SefA in orally and intraperitoneally infected mice; T cells from mice intraperitoneally immunised with live 11RX organisms and recombinant *aroA S.* Typhimurium SL3261 strains expressing SefA also proliferated and released cytokines *in vitro* when cultured with this antigen. BALB/c mice vaccinated orally with SefA using various antigen delivery systems based on strain SL3261 showed between 60-70% level of protection when challenged with the virulent 7314 strain, compared to 20% level of protection with strain SL3261 alone. A SefA mutant of 11RX was also protective (70%), compared to its isogenic parent which induced 80% level of protection. The results indicate that SefA contributes to, but is not the only antigen involved in inducing protection in mice against virulent challenge.

T cell epitope mapping of SefA was carried out, using an analogous antigen, the FanC subunit protein of the K99 fimbriae of enterotoxigenic *E. coli*, as a model. A recombinant approach involving the fusion of carboxyl-terminal truncations of FanC to alkaline phosphatase was adopted. The results indicate that the B cell epitopes of FanC are likely to be continuous. T cell epitope analysis of the fusions by *in vitro* T cell proliferation assays did not indicate the presence of any immunodominant epitope. However, T cell epitope mapping of SefA, using a series of overlapping 16 amino acid synthetic peptides of SefA, indicated an immunodominant T cell epitope of SefA in a region corresponding to amino acids 55-61 of the protein.

The implications of, and the propositions arising from, the results presented in this study, as well as potential areas of further research, are discussed.