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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 LD₅₀ 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.

CHAPTER 1	1
General Introduction	1
1.1 Microbe interactions with host	1
1.2 The Genus <i>Salmonella</i>	1
1.2.1 Description	1
1.2.2 Nomenclature	2
1.2.3 Epidemiology	3
1.2.4 <i>Salmonella</i> pathogenesis	6
1.2.4.1 Introduction	6
1.2.4.2 <i>Salmonella</i> -host cell interactions	7
1.2.4.3 Intracellular multiplication	9
1.2.4.4 Mechanisms of invasion by <i>Salmonella</i>	11
1.2.4.5 Signal transduction and membrane ruffling during entry of <i>Salmonella</i>	14
1.2.4.6 Environmental signals which influence entry of <i>Salmonella</i> into cells	15
1.3 Immunity to <i>Salmonella</i>	16
1.3.1 Introduction	16
1.3.2 Role of macrophages in immunity to <i>Salmonella</i>	16
1.3.3 Humoral immunity to <i>Salmonella</i>	18
1.3.4 Cell-mediated immunity to <i>Salmonella</i>	20
1.3.5 <i>Salmonella</i> immunogens and immunity	22
1.4 <i>Salmonella</i> Enteritidis	24
1.4.1 Introduction	24
1.4.2 Fimbriae of <i>S. Enteritidis</i>	25
1.4.3 Previous studies with <i>S. Enteritidis</i> 11RX	26

1.4.4 Aims of this thesis	28
CHAPTER 2	29
Materials and Methods	29
2.1 Animals	29
2.2 Tissue culture media and reagents	29
2.3 Bacteriological media	31
2.3.1 CBT medium	31
2.3.2 Luria Bertani (LB) broth and LB agar	31
2.3.3 Nutrient agar	31
2.3.4 Terrific broth	31
2.3.5 Minimal casein (Minca) medium	31
2.3.6 XLD medium	31
2.4 Enzymes	33
2.5 Antibiotics	33
2.6 Chemicals and reagents	34
2.7 Plasmids and cloning vectors	35
2.8 Oligodeoxynucleotides	36
2.9 Bacterial strains and antigens	37
2.9.1 Bacterial strains and their maintenance	37
Table 2.3 (continued)	38
2.9.2 Bacterial antigens and their preparation	39
2.10 Immunological methods	40
2.10.1 Immunisations	40

2.10.2 Lymphoid cell suspensions	40
2.10.3 Adherence to nylon wool columns	41
2.10.4 In vitro proliferation assays	41
2.10.5 T-cell phenotype analysis	42
2.10.6 Interferon Gamma (IFN- γ) assays	42
2.10.7 Interleukin-2 (IL-2) assays	42
2.10.8 Serology	43
2.10.9 DTH assays and measurement of footpad swelling	43
2.10.10 Preparation and absorption of anti-11RX serum	44
2.10.11 Preparation of anti-SEF14 monoclonal antibodies	45
2.10.12 Immunofluorescence microscopy	46
2.11 Transformation and electroporation of strains	47
2.11.1 Preparation and transformation of competent <i>E. coli</i> strains	47
2.11.2 Preparation of electrocompetent <i>Salmonella</i> strains	48
2.11.3 Electro-transformation and plating	48
2.12 DNA extraction procedures	49
2.12.1 Small scale plasmid isolation	49
2.12.2 Large scale plasmid isolation	50
2.13 Preparation of <i>S. Enteritidis</i> and <i>E. coli</i> genomic DNA	51
2.14 Analysis and manipulation of DNA	52
2.14.1 DNA quantitation	52
2.14.2 Restriction endonuclease digestion of DNA	52
2.14.3 Calculation of sizes of restriction fragments	52
2.14.4 Analytical and preparative separation of restriction fragments	53
2.14.5 Dephosphorylation of DNA with alkaline phosphatase	54

2.14.6 End-filling with Klenow fragment	55
2.14.7 <i>In vitro</i> cloning	55
2.14.7.1 Cosmid cloning	56
2.14.7.2 Construction of plasmid pYZ100	56
2.14.7.3 Cloning of <i>sefA</i> into pEV41b	57
2.14.8 Exonuclease deletion	58
2.14.9 <i>In vitro</i> transcription/translation	59
2.14.10 Polymerase Chain Reaction (PCR) Protocol	59
2.14.11 Labelling of DNA probes	60
2.14.11.1 Labelling of oligonucleotide #571	60
2.14.11.2 Labelling the 168 bp PCR product of <i>sefA</i>	60
2.14.12 DNA dot blotting with oligonucleotide probes	61
2.14.13 DNA hybridisation by Southern blotting	62
2.14.14 Sequencing using dye-labelled primers	63
2.14.15 Sequencing with dye-labelled terminators	64
2.14.16 Analysis of DNA sequences	65
2.15 Protein analysis	66
2.15.1 Preparation and purification of SEF14 protein	66
2.15.2 Preparation of SEF14 for N-terminal sequencing	67
2.15.3 Preparation of whole cell lysates of bacteria	67
2.15.4 Cell fractionation	68
2.15.5 Protein overproduction	69
2.15.5.1 Overproduction of FanC:: <i>PhoA</i> fusions	69
2.15.5.2 Overproduction of SefA protein	69
2.15.6 SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)	70

2.15.7 Colony (dot) blotting and Western blotting	70
2.15.8 Electron microscopy	71
2.15.8.1 Preparation of protein A-gold	71
2.15.8.2 Immuno-electron microscopy	71
2.15.9 Determination of protein concentration	72
2.16 LPS (silver) staining	72
2.17 <i>In vivo</i> studies	73
2.17.1 Colonisation and competition studies.	73
2.17.2 LD ₅₀ measurements	73
2.17.3 Protection studies	74
2.18 <i>In vitro</i> adherence and invasion studies	74
2.19 Measurement of bacterial growth in HeLa cells	76
2.20 Double immunofluorescence microscopy	76
CHAPTER 3	78
Preliminary characterisation of SefA	78
3.1 Introduction	78
3.2 Results	79
3.2.1 N-terminal amino acid sequence of the immunogenic protein	79
3.2.2 Purification of SEF14	80
3.2.3 <i>In vitro</i> response of normal and 11RX-primed T cells to SEF14	81
3.2.4 DTH responses	84
3.2.5 Preparation of anti-11RX serum	84
3.2.6 Characterisation of monoclonal antibodies to SEF14	85

3.2.7 Immunogold electron microscopy	88
3.3 Discussion	89
CHAPTER 4	93
The role of SefA in the pathogenesis of <i>S. Enteritidis</i>	93
4.1 Introduction	93
4.2 Results	94
4.2.1 Cosmid cloning	94
4.2.2 Southern hybridisation and restriction map construction	96
4.2.3 Nucleotide sequence of <i>sefA</i>	98
4.2.4 Construction of <i>sefA</i> complementing plasmid	100
4.2.5 Construction and analysis of <i>sefA</i> mutants	100
4.2.6 Colonisation studies	106
4.2.7 <i>In vitro</i> adherence and invasion of HeLa cells	109
4.3 Discussion	113
CHAPTER 5	117
SefA protein is a protective antigen of <i>S. Enteritidis</i>	117
5.1 Introduction	117
5.2 Results	119
5.2.1 Production of SefA by Salmonella vaccine strains.	119
5.2.2 Oral immunisation and protection	121
5.2.3 Antibody responses to SefA	121
5.2.4 <i>In vitro</i> responses of primed T cells to various Ags	123
5.2.5 Cytokine assays	124

5.2.6 Protection after oral challenge	125
5.3 Discussion	126
CHAPTER 6	130
Epitope analysis of FanC and SefA	130
6.1 Introduction	130
6.2 Results and Discussion	130
6.2.1 Determination of C-terminal deletion end-points in <i>fanC</i> .	133
6.2.2 Overproduction of FanC::PhoA fusions.	134
6.2.3 Epitope analysis of FanC.	137
6.2.3.1 B cell epitope mapping of FanC	137
6.2.3.2 T cell epitope mapping of FanC	139
6.2.4 Overproduction of SefA	141
6.2.5 Epitope analysis of SefA	142
6.2.5.1 SefA peptides are not produced by C-terminal deletions of <i>sefA</i>	142
6.2.5.2 Use of synthetic peptides to define T-cell epitopes of SefA	144
6.3 Summary	147
CHAPTER 7	150
Summary and conclusions	150
7.1 Previous studies	150
7.2 Current work and perspectives	151