Characterization of the O antigen gene cluster and structural analysis of the O antigen of *Francisella tularensis* subsp. *tularensis*

Joann L. Prior,¹ Richard G. Prior,¹ Paul G. Hitchen,² Helen Diaper,¹ Kate F. Griffin,¹ Howard R. Morris,^{2,3} Anne Dell² and Richard W. Titball^{1,4}

Correspondence

Joann L. Prior
JLPRIOR@dstl.gov.uk

¹Dstl Porton Down, Salisbury, Wiltshire SP4 0JQ, UK

²Department of Biological Sciences, Imperial College, London SW7 2AZ, UK

³M-SCAN Mass Spectrometry Research and Training Centre, Silwood Park, Ascot SL5 7PZ, UK

⁴Department of Infectious and Tropical Disease, London School of Hygiene and Tropical Medicine, Keppel St., London WC1E 7HT, UK

A gene cluster encoding enzymes involved in LPS O antigen biosynthesis was identified from the partial genome sequence of *Francisella tularensis* subsp. *tularensis* Schu S4. All of the genes within the cluster were assigned putative functions based on sequence similarity with genes from O antigen biosynthetic clusters from other bacteria. Ten pairs of overlapping primers were designed to amplify the O antigen biosynthetic cluster by PCR from nine strains of *F. tularensis*. Although the gene cluster was present in all strains, there was a size difference in one of the PCR products between subsp. *tularensis* strains and subsp. *holarctica* strains. LPS was purified from *F. tularensis* subsp. *tularensis* Schu S4 and the O antigen was shown by mass spectrometry to have a structure similar to that of *F. tularensis* subsp. *holarctica* strain 15. When LPS from *F. tularensis* subsp. *tularensis* Schu S4 was used to immunize mice that were then challenged with *F. tularensis* subsp. *tularensis* Schu S4, an extended time to death was observed.

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INTRODUCTION

Francisella tularensis is a small, Gram-negative coccobacillus that causes the zoonotic disease tularemia. Tularemia occurs in the Northern hemisphere, with cases reported frequently in Europe, North America, Asia, northern Russia and Japan. Rodents are thought to be the main reservoir of the bacterium, with ticks as one of the main vectors (Stewart, 1998). The genus *Francisella* includes *F. tularensis*, *Francisella novicida* and *Francisella philomiragia*. *F. tularensis* has been divided into three subspecies (*tularensis*, *holarctica* and *mediaasiatica*) on the basis of virulence, citrulline ureidase activity and acid production from glycerol (Olsufjev & Meshcheryakova, 1983; Olsufjev *et al.*, 1959). In addition, it has been suggested that *F. novicida* should also be considered a subspecies of *F. tularensis* (Hollis *et al.*, 1989). Strains of *F. tularensis* subsp. *tularensis* are highly virulent in

humans, whilst strains belonging to *F. tularensis* subsp. *holarctica* and subsp. *mediaasiatica* cause a milder form of the disease.

There have been some limited studies on the structure and immunogenicity of LPS isolated from *F. tularensis* subsp. *holarctica* (Dreisbach *et al.*, 2000). It is reported that the LPS is less toxic than other Gram-negative LPS (Ancuta *et al.*, 1996; Sandstrom *et al.*, 1992) and that its O antigen contains the rare sugars 2-acetamido-2,6-dideoxy-D-glucose (QuiNAc) and 4,6-dideoxy-4-formamido-D-glucose (Qui4NFm) and two moles of 2-acetamido-2-deoxy-D-galacturonamide (GalNAcAN), to give the repeat structure 4- α -GalNAcAN-1,4- α -GalNAcAN-1,3- β -QuiNAc-1,2- β -Qui4NFm (Vinogradov *et al.*, 1991). The repeat structure is similar to those found in the O antigens of *Pseudomonas aeruginosa* O6 and *Shigella dysenteriae* type 7 (Knirel *et al.*, 1988; Vinogradov *et al.*, 1987). However, there are no reports of LPS characterization from highly virulent (subsp. *tularensis*) strains of *F. tularensis*. It is known that animals immunized with LPS from *F. tularensis* subsp. *holarctica* are fully protected against a subsequent challenge with a subsp. *holarctica* strain (Fulop *et al.*, 1995), but not against challenge with a subsp. *tularensis* strain (Fulop *et al.*, 2001). This raises

Abbreviations: GalNAcAN, 2-acetamido-2-deoxy-D-galacturonamide; QuiNAc, 2-acetamido-2,6-dideoxy-D-glucose; Qui4NFm, 4,6-dideoxy-4-formamido-D-glucose.

The GenBank/EMBL/DDBJ accession number for the sequence of the *F. tularensis* Schu S4 O antigen gene cluster is AY217763.

the possibility that the LPS from a subsp. *holarctica* strain is not identical to that of a subsp. *tularensis* strain. In order to investigate this, we have isolated LPS from *F. tularensis* strains Schu S4 (subsp. *tularensis*) and LVS (subsp. *holarctica*) and compared their structure. We also aimed to elucidate the genetic basis of O antigen expression in *F. tularensis* strain Schu S4 and to use this information to investigate the distribution of the O antigen gene cluster in different strains of *F. tularensis* subsp. *tularensis* and subsp. *holarctica*.

METHODS

Bacterial strains and growth conditions. The following strains were used: *F. tularensis* subsp. *tularensis* strains Schu S4, 199 and 041; *F. tularensis* subsp. *holarctica* strains LVS, 200, 025, 075 and HN63. Strains were cultured at 37 °C on BCGA agar for 48 h.

LPS purification. LPS was purified from *F. tularensis* subsp. *tularensis* Schu S4 and *F. tularensis* subsp. *holarctica* LVS using a hot-phenol and water extraction method (Westphal & Jann, 1965). In brief, LPS was extracted from freeze-dried bacteria with 45 % phenol at 67 °C. The mixture was stirred for 30 min and centrifuged. The resulting pellet was re-extracted and the water phases from the two extractions were dialysed against water for 3 days. The resulting solution was ultracentrifuged and treated with RNase and proteinase K.

Gel electrophoresis and silver staining. Glycine gel electrophoresis was performed according to the buffer system of Laemmli (1970) using a 12.5 % separating gel with a 4.5 % stacking gel. Aliquots (10 µl) of each sample were electrophoresed for approx. 2 h at 100 mV in the Mini-protein II slab system (Bio-Rad). Gels were silver-stained according to the method of Chart (1994), with the oxidation step increased to 10 min. To achieve comparable staining, forty times more *F. tularensis* LPS was loaded than *Escherichia coli* K325 LPS.

Nucleotide sequence analysis. The sequence encoding the O antigen biosynthetic cluster was identified from the *F. tularensis* Schu S4 partial genome sequence (Prior *et al.*, 2001), available at <http://artedi.ebc.uu.se/Projects/Francisella/>. Known protein sequences (obtained from GenBank) involved in the biosynthesis of the O antigen of other bacteria were used to probe the partial genome sequence data using TBLASTN (Altschul *et al.*, 1997). The contig containing the putative O antigen gene cluster was extracted and subsequently analysed using the annotation tool ARTEMIS (<http://www.sanger.ac.uk/Software/Artemis>).

The protein sequences encoded by the putative O-antigen flippase gene (*wzx*) and O-antigen polymerase gene (*wzy*) were analysed for trans-membrane helices using TMHMM (Sonnhammer *et al.*, 1998).

PCR analysis of the putative O antigen gene cluster. DNA was prepared from the *F. tularensis* strains listed above by phenol extraction as described by Karlsson *et al.* (2000). Ten pairs of overlapping PCR primers were designed using the DNASTar program PrimerSelect to amplify the whole of the putative O antigen gene cluster in approximate 2 kb segments. The primers were designed with annealing temperatures ranging from 42 to 59 °C, although all were used successfully at 49 °C.

PCR amplification using each pair of primers with each template DNA was carried out in the following mixture: 1× PCR buffer (including 1.5 mM MgCl₂), 0.2 mM dNTPs, 2.5 mM forward primer, 2.5 mM reverse primer, 2.0 µl template DNA, 0.5 U *Taq* polymerase and filtered sterile water to a final volume of 20 µl. The reaction mixtures were incubated at 90 °C for 1 min and then cycled at 90 °C for 1 min, 49 °C for 1 min and 72 °C for 145 s for 30 cycles, with a final incubation at 72 °C for 10 min.

PCR products were visualized on 0.5 % agarose gels, with ethidium bromide staining. PCR buffer, dNTPs and polymerase were from Roche. PCR primers were synthesized by MWG-Biotech.

Cloning of PCR products. PCR products amplified from Schu S4, HN63 and LVS DNA using primer pair 8 were cloned into pGEM-T easy (Promega) for sequence analysis. Ligated DNA was transformed in *E. coli* JM109 chemically competent cells (Promega) and putative clones were screened using both colony PCR and digestion with restriction endonucleases. All DNA manipulations, including ligations, transformations, colony PCR, restriction endonuclease digestions and agarose gel electrophoresis, were carried out according to standard methods (Sambrook *et al.*, 1989). Purification of PCR products from agarose gel was achieved using the QIAquick gel extraction kit (Qiagen) according to the manufacturer's instructions.

The three constructs were sequenced using the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977) with universal primers. Each sequence was compared and the BLAST (Altschul *et al.*, 1990) function of the ARTEMIS software package was used for similarity searches in the locally held GenBank databases to identify the functions of the different regions of DNA.

Mass spectrometric (MS) analysis of the O antigen molecule.

Polysaccharide was released in aqueous 1 % acetic acid at 100 °C for 2 h and then reduced with NaBH₄ (10 mg ml⁻¹) in 2 M NH₃ for 2 h at room temperature (Hitchen *et al.*, 2002). Excess borates were removed by repeated additions (×4) of 10 % acetic acid in methanol. Methylation using the sodium hydroxide procedure was performed as described by Dell *et al.* (1994). After derivatization, the reaction products were purified on a Sep-Pak C₁₈ column (Waters) as described by Dell *et al.* (1994).

Matrix-assisted laser desorption/ionization (MALDI)-MS was performed on a Perseptive Biosystems Voyager Elite mass spectrometer with delayed extraction. Samples were dissolved in 200 µl methanol/water (80:20, v/v) and aliquots (0.5 µl) of the resulting solutions were analysed using a matrix of 2,5-dihydroxybenzoic acid. Angiotensin and insulin B chain were external calibrants.

Immunization with LPS and protection studies. The ability of *F. tularensis* subsp. *tularensis* Schu S4 LPS to protect BALB/c mice from a *F. tularensis* subsp. *tularensis* challenge was determined by immunizing a group of six female BALB/c mice (Charles River Laboratories) with LPS purified as above. On days 0, 7 and 14, mice were dosed intraperitoneally with 50 µg LPS in PBS. They were challenged 21 days after the last immunization with 10 c.f.u. *F. tularensis* subsp. *tularensis* Schu S4 by the subcutaneous route, delivered in 0.1 ml. All procedures were carried out in accordance with Home Office guidelines.

RESULTS AND DISCUSSION

LPS purification

The hot-phenol/water extraction method was used to purify LPS from 2.2 g freeze-dried cells of *F. tularensis* subsp. *tularensis* Schu S4. This resulted in 7 mg LPS, a yield of 0.3 %. LPS from *F. tularensis* subsp. *holarctica* LVS was extracted from 1.25 g freeze-dried bacteria using the same method. This resulted in 2 mg LPS, a yield of 0.16 %. To obtain visible silver-staining of LPS after SDS-PAGE, the oxidation step was increased from 5 to 10 min (Fig. 1).

Fig. 1. SDS-PAGE of LPS isolated from various strains. Lanes: 1, *E. coli* strain K325 (1.25 µg); 2, *F. tularensis* subsp. *holarctica* LVS (50 µg); 3, *F. tularensis* subsp. *tularensis* Schu S4 (50 µg).

MS analysis of the O antigen molecule

The composition of the O antigen expressed by *F. tularensis* subsp. *tularensis* Schu S4 was determined by MS in order to allow direct comparison with the previously reported composition of the O antigen from *F. tularensis* subsp. *holarctica* strain 15. Mild hydrolysis was used to cleave lipid A from the core glycan. The products of this hydrolysis are expected to be free core and O antigen-substituted core of varying O antigen chain length, together with lipid A. Lipid A was not observed in the following MS experiments because it was separated from the free glycans during the Sep-Pak purification stage.

Samples were reduced and methylated prior to MALDI-MS analyses and data attributable to the derivatized O antigen plus core oligosaccharide are shown in Fig. 2. The data suggest that there are two core structures. The larger core gives $[M+Na]^+$ signals at m/z 1422 and 1376, corresponding to the composition HexNAcHex₄Kdo. The signals at m/z 1218 and 1172 correspond to core containing one fewer hexose residue (i.e. HexNAcHex₃Kdo). The signals at m/z 1376 and 1172 are -46 satellites from m/z 1422 and 1218, respectively, which we have previously shown to be diagnostic of oligosaccharides containing a reducing Kdo residue (Dell *et al.*, 1990) expected for the products of mild acid hydrolysis of LPS. Previous work by Vinogradov *et al.* (1991) has shown that the O antigen repeat unit of *F. tularensis* subsp. *holarctica* strain 15 has the composition QuiNAc, Qui4NFm and two moles of GalNAcAN. The signals at m/z 2382 $[M+Na]^+$ and m/z 2336 (-46 satellite) are consistent with the larger core (HexNAcHex₄Kdo) plus one O antigen repeat unit, where the O antigen unit has the composition QuiNAc(GalNAcAN)₂Qui4NFm. An additional one and two O antigen repeat units would give rise to the signals at m/z 3342 and 4302, respectively. Clusters consistent with one and two further repeat units are visible above the base line (Fig. 2 inset; m/z 5262 and 6222). The two spectra (Fig. 2a, b) show remarkably similar data. These data suggest that the two strains have identical O antigen repeats.

Fig. 2. MALDI-MS of methylated polysaccharide from *F. tularensis* strains Schu4 (a) and LVS (b). Polysaccharides were released by mild acid hydrolysis, reduced and methylated. Derivatized polysaccharide was purified by Sep-Pak and the 75% (v/v) aqueous acetonitrile fractions were analysed by MALDI-MS.

Analysis of *F. tularensis* putative O antigen biosynthetic gene cluster

The *F. tularensis* putative O antigen biosynthetic gene cluster was found to be approximately 17 kb in length and was predicted to contain 15 genes involved in O antigen biosynthesis (Table 1). The cluster was flanked by two transposases (Fig. 3), raising the possibility that the cluster may have been horizontally acquired. The genes *manC* and *manB* were located downstream of the second transposase and are probably not involved in biosynthesis of the O antigen, as mannose was not found to be a component of the *F. tularensis* O antigen, nor is it likely to be one of the intermediate products required for its synthesis. The *manC* and *manB* genes may have been involved in biosynthesis of the O antigen in an ancestor of *F. tularensis*.

Fig. 3 also shows the G+C content plot of the cluster, using a window size of 500 bases. The overall G+C content of this region (31.27%) was slightly lower than the mean genome content of approximately 33% (Prior *et al.*, 2001). The

Table 1. Putative O antigen genes and gene products of *F. tularensis* subsp. *tularensis* Schu S4

Similar protein matches are based on the highest identity across the whole protein.

Putative gene product	Length (aa)	Putative function	Similar protein	Identity (%)	Organism	Accession no.
Transposase	247	Hypothetical protein		37.1	<i>Mesorhizobium loti</i>	BAB53737
		Transposase		35.2	<i>Vibrio salmonicida</i>	CAC35348
WbtA	578	Sugar epimerase	PglF	46.8	<i>Campylobacter jejuni</i>	AAD51388
			WbpM	41.7	<i>Pseudomonas aeruginosa</i>	AAD45269
WbtB	205	Galactosyl transferase	Cps9F	64.7	<i>Streptococcus suis</i>	AAF18949
		Glycosyl transferase		41.3	<i>Pseudomonas aeruginosa</i>	H83366
WbtC	263	UDP-glucose 4-epimerase		47.5	<i>Bacillus halodurans</i>	BAB07434
			WbpV	32.8	<i>Pseudomonas aeruginosa</i>	AAF23991
WbtD	363	Sugar transferase		39.3	<i>Streptococcus pneumoniae</i>	CAB05923
			WbpU	30.4	<i>Pseudomonas aeruginosa</i>	AAF23992
WbtE	436	LPS biosynthesis	WbgT	53.6	<i>Shigella sonnei</i>	T44520
		Dehydrogenase	WbpO	52.8	<i>Pseudomonas aeruginosa</i>	AAF23997
WbtF	323	C 4-epimerase	WbpP	51.2	<i>Pseudomonas aeruginosa</i>	AAF23998
Wzy	409	Membrane protein/O-antigen polymerase		24.6	<i>Clostridium acetobutylicum</i>	AAK81105
WbtG	366	Transferase	WbpT	27.9	<i>Pseudomonas aeruginosa</i>	AAF23993
WbtH	628	Asparagine synthetase	WbpS	48.5	<i>Pseudomonas aeruginosa</i>	AAF24002
WbtI	360	Sugar transaminase/perosamine synthetase		59.9	<i>Clostridium acetobutylicum</i>	AAK80306
				43.1	<i>Pseudomonas aeruginosa</i>	AAK15326
WbtJ	241	Formyl transferase	Unnamed	40.4	<i>Mycobacterium tuberculosis</i>	AAK47850
Wzx	495	O-Antigen flippase	Wzx	28.9	<i>Escherichia coli</i>	AAI67558
WbtK	286	Glycosyl transferase		36.7	<i>Clostridium acetobutylicum</i>	AAK80304
WbtL	294	Glucose-1-phosphate thymidyltransferase	Unnamed	67.7	<i>Actinobacillus actinomycetemcomitans</i>	T00103
WbtM	348	dTDP-D-glucose 4,6-dehydratase	RmlB	63.9	<i>Legionella pneumophila</i>	CAB65206
		dTDP-D-glucose 4,6-dehydratase		56.3	<i>Pseudomonas aeruginosa</i>	B83000
Transposase	126	Transposase		29.7	<i>Streptococcus pneumoniae</i>	AAK76082
ManC	468	Mannose-1-phosphate guanylyltransferase	ManC	50.4	<i>Vibrio cholerae</i>	Q07024
ManB	494	Phosphomannomutase	ManB	45.9	<i>Salmonella choleraesuis</i>	Q00330

Fig. 3. Genetic organization of the O antigen gene cluster in *F. tularensis* subsp. *tularensis* Schu S4.

central section of the cluster, from *wzy* to *wbtK*, had an even lower G+C content. Downstream from *manC*, on the opposite strand, the genes for the transcription termination factor *rho* and thioredoxin were identified.

The O antigen repeat unit of *F. tularensis* (Vinogradov *et al.*, 1991) is shown in Fig. 4, together with the putative role of the genes involved in O antigen biosynthesis. Based on homology to other LPS and sugar biosynthetic genes, in particular to genes from *P. aeruginosa* serotype O6, which expresses a similar O antigen repeat structure (Knirel *et al.*, 1985), the putative roles of the gene products have been assigned. It is proposed that the biosynthesis of QuiNAc involves WbtA, a dehydratase and WbtC, which showed sequence similarity to known UDP-glucose 4-epimerases. WbtA and WbtC showed sequence similarity to WbpM and WbpV of *P. aeruginosa* strain O6. Both of these enzymes are thought to be involved in QuiNAc biosynthesis and have been shown to be essential for O antigen synthesis in *P. aeruginosa* strain O6 (Belanger *et al.*, 1999). WbtE, WbtF and WbtH are proposed to be involved in GalNAcAN biosynthesis. WbtF showed sequence similarity to known UDP-glucose 4-epimerases, including WbpP in *P. aeruginosa* and VipB in *Salmonella typhi*. WbtE showed sequence similarity to WbpO and VipA, which are UDP-GalNAc dehydrogenases involved in the formation of 2-acetamido-2-deoxy-D-galactouronic acid (GalNAcA) in

P. aeruginosa and *Salmonella typhi*, respectively (Hashimoto *et al.*, 1993; Zhao *et al.*, 2000). WbtH had sequence similarity to glutamine amidotransferases, including WbpS of *P. aeruginosa* serotype O6, which may be involved in the formation of the GalNAcAN amido group. Biosynthesis of the fourth sugar, Qui4NFm, probably requires WbtI, WbtJ, WbtL and WbtM. Sequence similarity suggests that WbtL may be involved in the formation of the activated sugar dTDP-D-glucose, with WbtM functioning as a dTDP-D-glucose 4,6-dehydratase. WbtI is proposed to be involved in Qui4NFm amination, since it shows sequence similarity to RfbE from *Vibrio cholerae*, a perosamine synthetase (Albermann & Piepersberg, 2001). Finally, WbtJ is likely to be responsible for the addition of the N-formyl moiety, showing significant sequence similarity to formyltransferases.

Specific glycosyltransferases are required to form the oligosaccharide units of the O antigen repeat. Four glycosyltransferases would be necessary for the synthesis of each O antigen unit in *F. tularensis*. Based on sequence similarity to known glycosyltransferases, WbtB is proposed to mediate the addition of QuiNAc to undecaprenyl phosphate (Und-P) to initiate O antigen biosynthesis. WbtD and WbtG are probable GalNAcAN transferases, possibly involved in the addition of the two consecutive GalNAcAN residues onto the O antigen unit. WbtD shows sequence similarity to WbpU of *P. aeruginosa* strain O6, proposed to transfer 2-formamido-2-deoxy-D-galactouronamide (GalNFmAN) onto QuiNAc (Belanger *et al.*, 1999). WbtG shows sequence similarity to WbpT of *P. aeruginosa*, which is thought to be involved in addition of GalNAcA to GalNFmAN (Belanger *et al.*, 1999). WbtK is probably the fourth glycosyltransferase, which adds 4,6-dideoxy-4-formamido-D-glucose (QuiNA4Fm) to complete the tetrasaccharide O unit.

Fig. 4. Schematic structure of an O antigen subunit of *F. tularensis* subsp. *tularensis* Schu S4 and the assignment of putative functions to the predicted products of the O antigen gene cluster. A single O-unit is shown, with sugar residues and glycosidic linkages indicated. QuiNAc4Fm, 4,6-dideoxy-4-formamido-D-glucose; GalNAcAN, 2-acetamido-2-deoxy-D-galactouronamide; QuiNAc, 2-acetamido-2,6-dideoxy-D-glucose.

Within the *F. tularensis* O antigen gene cluster, there were no polynucleotide repeats associated with slipped-strand mispairing that could explain the phase variation of LPS observed by other workers (Cowley *et al.*, 1996).

There are two main O antigen synthesis modes, O-antigen polymerase (*wzy*)-dependent and *wzy*-independent. Two genes in the *F. tularensis* O antigen gene cluster would

encode proteins with a high degree of sequence similarity to Wzy and Wzx, suggesting that transportation and polymerization of the O antigen is via a wzy-dependent pathway.

The inability to identify a gene that could encode a Wzz homologue, an O antigen chain length regulator, probably means that the chain length is determined by some other mechanism in *F. tularensis*. This may not be unusual, as many bacterial species have an LPS and O antigen that would require wzy-dependent synthesis, but Wzz homologues have not been identified in all of them.

PCR analysis of the O antigen gene cluster

To determine whether the O antigen gene cluster was present in different strains of *F. tularensis* subsp. *tularensis* and subsp. *holarctica*, ten pairs of PCR primers were designed in order to amplify overlapping regions of the putative O antigen gene cluster. Nine of the PCR products (derived using primer sets 1–7, 9 and 10) from each template DNA appeared to be the same size in all strains when examined by agarose gel electrophoresis (not shown).

PCR using primer pair 8 revealed a difference in size between the DNA fragments amplified from subsp. *tularensis* strains and subsp. *holarctica*. These fragments were cloned and sequence analysis of this region from the subsp. *tularensis* strain Schu S4 and the subsp. *holarctica* strains HN63 and LVS showed that the deletion in Schu S4 occurs at the beginning of a putative transposase that is similar to IS630-sp1 transposase ORF 1 of *Streptococcus pneumoniae*. The subsp. *tularensis* strains show a deletion of 303 nucleotides when compared with subsp. *holarctica* strains (including LVS). The overall similarity between the subsp. *tularensis* and subsp. *holarctica* clusters seems to indicate that the transposase insertion took place in *F. tularensis* before division of the subspecies. Partial deletion of the transposase would have the effect of stabilizing this region of DNA, as this enzyme is necessary for insertion events to take place (Rubin *et al.*, 2001; Sangari *et al.*, 2000). The difference in this region could be utilized to devise diagnostic systems for discriminating subsp. *tularensis* from subsp. *holarctica* strains.

Protection studies

When animals were immunized with LPS extracted from *F. tularensis* subsp. *holarctica* strain LVS and challenged with *F. tularensis* subsp. *tularensis* strain Schu S4, no protection was observed (Fulop *et al.*, 2001). To determine whether this was due to a difference in LPS composition, mice were immunized with *F. tularensis* subsp. *tularensis* Schu S4 LPS and challenged with *F. tularensis* subsp. *tularensis* Schu S4. At the challenge dose of 10 c.f.u. (10 MLD), the immunized group demonstrated a significantly ($P = 0.01$) delayed time to death (mean increase of 64 h) compared with the unimmunized control group. Work carried out previously by Fulop *et al.* (2001) with mice immunized with LPS from *F. tularensis* subsp. *holarctica* LVS produced similar results, with an extended time to death of 24 h when animals were subsequently challenged with 10 c.f.u. *F. tularensis* subsp.

tularensis Schu S4. These findings indicate that the LPS from *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* have similar immunological properties.

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