Structural analysis of the O-antigen of *Francisella tularensis* subspecies *tularensis* strain OSU 10

*Francisella tularensis*, the causative agent of the disease tularaemia, is one of the most infectious bacteria known. *F. tularensis* subspecies *tularensis* (type A) and *holarctica* (type B) are the major subspecies, with the former being highly virulent for humans (Conlan et al., 2002). A live vaccine strain (LVS) of *F. tularensis* empirically derived from a virulent strain of type B *F. tularensis* provides considerable protection against highly virulent type A strains in humans (Conlan et al., 2002; Fulop et al., 2001). Although the *F. tularensis* LVS is attenuated for humans, it is fully virulent for mice (Conlan et al., 2002), and infection with *F. tularensis* LVS in mice has been used as an experimental model of tularaemia in a number of studies.

Immunization of mice with LPS derived from *F. tularensis* LVS induced protection against intraperitoneal challenge with the LVS but not against a virulent strain of type A *F. tularensis*. However, the immunization significantly increased the survival time in mice challenged with the virulent strain of type A *F. tularensis* (Fulop et al., 2001). Similarly, mice vaccinated with O-antigen of the LPS from *F. tularensis* LVS chemically conjugated to BSA were protected against an intradermal challenge with a highly virulent strain of type B *F. tularensis* but not against a virulent type A strain (Conlan et al., 2002). mAbs directed against O-antigen and core polysaccharide of the LPS from *F. tularensis* LVS recognized both type A and type B strains (Fulop et al., 1991). This suggests the presence of common epitopes in the LPS of both subspecies. However, the mouse protection studies suggest possible differences in the structure of LPS/O-antigens between the two subspecies (Fulop et al., 2001; Prior et al., 2003). Conversely, different mechanisms of protection may be required to resolve infections caused by the two subspecies (Fulop et al., 2001).

The O-antigen structure of *F. tularensis* strain 15 (a vaccine strain derived from type B *F. tularensis* in the former Soviet Union) was determined to contain repeating tetrasaccharide subunits, 4-(α-D-GalpNAcAN-(1-4)-α-D-GalpNAcAN-(1-3)-β-D-QuipNAc-(1-2)-β-D-Quip4NFo-1), using 1H- and 13C-NMR spectroscopy (Vinogradov et al., 1991). *Francisella tularensis* LVS was also found to express O-antigen identical to that of *F. tularensis* strain 15 (Conlan et al., 2002). Studies on the structure of O-antigen from virulent strains of type A *F. tularensis* have been limited to the Schu S4 strain, which is a virulent but highly passaged laboratory strain (Prior et al., 2003). The repeating units of the O-antigens from the Schu S4 strain and the LVS were presumed to be the same based on MALDI (matrix-assisted laser desorption/ionization)-MS analysis (Prior et al., 2003). In the present study we report the 1H- and 13C-NMR spectroscopy structural analysis of O-antigen from a field strain of type *F. tularensis* (strain OSU 10).

*F. tularensis* subspecies *tularensis* strain OSU 10 was isolated from a cat that died of tularaemia and subspecies identification was based on Biolog metabolic fingerprinting (Biolog) and PCR (Petersen et al., 2004) and confirmed by the Centers for Disease Control (Atlanta, GA, USA). LPS from strain OSU 10 was isolated using the Tri-reagent method described elsewhere (Yi & Hackett, 2000), and analysed by SDS-PAGE and Western blotting. LPS samples were run on 12% acrylamide gels followed by silver staining. Isolated LPS showed the characteristic ladder pattern on acrylamide gels, indicating variation in the polysaccharide side-chain length (Fig. 1a). Further, the LPS was reactive with mAbs specific for *F. tularensis* O-antigen (BioDesign; Fig. 1b).

The O-antigen was isolated from type A *F. tularensis* strain OSU 10 LPS by acid hydrolysis. Briefly, LPS was suspended in 1% acetic acid and heated at 100°C for 2-5 h, the lipid portion was removed by centrifugation at 12 000 g for 20 min, and the supernatant containing O-antigen was freeze-dried. The O-antigen was further purified by gel filtration chromatography. Lyophilized O-antigen was suspended in 50 mM ammonium acetate and passed through a Toyopearl HW-50F (Supelco) gel filtration column. Elution of polysaccharide was monitored by refractive index. The three major fractions were pooled separately and freeze-dried. The O-antigen fraction, eluting just after the void volume, was identified by its immunoreactivity with mAbs specific for *F. tularensis* O-antigen and by its 1H NMR spectrum (Fig. 2). A two-dimensional (2D) 1H-13C heteronuclear multiple-quantum coherence (HMQC) spectrum of the O-antigen was obtained on a Varian Inova 600 NMR spectrometer at 30°C with continuous wave water presaturation using the standard pulse sequence on the instrument.

The 1H NMR spectrum of O-antigen from type A *F. tularensis* strain OSU 10 was almost identical to that shown for the O-antigen of *F. tularensis* LVS (Fig. 2; Conlan et al., 2002), except for some low-intensity signals between 3.7 and 4 p.p.m., indicating a small amount of contamination by another polysaccharide (see below). For a more definitive check on the identity between the O-antigen from the two subspecies...
The NMR data suggest that type A *F. tularensis* strain OSU 10 has an identical O-antigen to that of type B *F. tularensis* strain 15 and *F. tularensis* LVS. The present study supports the theory that strains of both type A and type B *F. tularensis* have identical O-antigen repeats (Vinogradov & Perry, 2004). Further analysis of O-antigen structures from additional strains should confirm this. Thus, it appears that different mechanisms of protection are required to resolve infections caused by virulent strains of type A and type B *F. tularensis* (Fulop et al., 2001).

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