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

Francisella tularensis, the causative agent of the disease tularemia, 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 tularemia 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 from F. tularensis LVS chemically conjugated to BSA were protected against an intradermal challenge with a highly virulent strain of type B F. tularensis. However, the immunization was not effective 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 A F. tularensis (strain OSU 10).

F. tularensis subspecies tularensis strain OSU 10 was isolated from a cat that died of tularemia 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 (BioDesign; Fig. 1b). The 1H NMR spectrum of O-antigen from F. tularensis strain OSU 10 LPS developed with mAbs specific to F. tularensis O-antigen. 

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.
subspecies of *F. tularensis* the HMQC spectrum of the type A *F. tularensis* strain OSU 10 O-antigen was compared to the HSQC (heteronuclear single-quantum coherence) spectrum reported for *F. tularensis* LVS (Fig. 2; Conlan *et al.*, 2002). Both types of 2D spectra correlate chemical shifts of directly bonded carbons and protons. All of the signals present in the LVS strain were present at the same carbon and proton chemical shifts in the strain OSU 10, indicating that the structures of the two polysaccharides are the same. The signal for the C-1, H-1 of the N-acetylquinovosamine was rather weak because it was under the water peak that had been suppressed. Some additional signals were observed in the HMQC spectrum of strain OSU 10. These probably came from a small amount of contamination with the glucans reported earlier (Conlan *et al.*, 2003). Judging from the low intensity of the signals in the one-dimensional (1D) spectrum, corresponding to the putative C-6, H-6 signal at 61 p.p.m., 3-9 p.p.m. for glucose, the contamination is quite minor.

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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