S.19 STRUCTURAL ANALYSIS OF GLYCOCONJUGATES: GLYCOLIPIDS

S19.1

Structural Analysis of the Endotoxically Active Lipid A Component of the Halophilic Bacterium *Rhodospirillum salinarum*

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The structural elucidation of lipid A, isolated from the cell wall lipopolysaccharide of *Rhodospirillum salinarum*, by chemical methods including laser desorption mass spectrometry, revealed that we were dealing with a mixed lipid A-type (1) being composed of three different 1,4′-bisphosphorylated β(1 – 6)-linked backbone hexosaminyl-hexosamine-disaccharides, namely of GlcN – GlcN, 2,3-diamino-2,3-dideoxy-D-Glc – (DAG)-DAG, and DAG – GlcN. The latter mentioned hybrid type, i.e. the 2,3-diamino-2,3-dideoxy-β-D-glucopyranosyl-(1 – 6)-D-glucosamine disaccharide was hitherto reported only once by Moran *et al.* (2) from LPS of the serotype O:2 of the pathogenic Gram-negative species *Campylobacter jejuni*. In contrast to the latter, the lipid A of *R. salinarum* contained preferentially 3-OH-14:0 and 3-OH-18:0 (molar ratio about 1:4) as amide-linked, and 18:1 and 19:1 (molar ratio about 1:1) as ester-linked fatty acids. The mass spectra of the liberated acyl-oxyacyl residues proved the concomitant presence of 3-O-(19:1)-14:0 and 3-O-(18:1)-18:0 as the predominant amide-linked diesters in this lipid A. The glycosidically, as well as the ester-linked phosphate groups, were not substituted by ETN, P-ETN, or by 4-amino-4-deoxy-L-arabinose, in contrast to most enterobacterial lipid As.

LPS of *R. salinarum* showed a lethality (in $C57Bl_{10}$ SCSN-mice) in a range of 1/10 – 1/100 from that reported for *Salmonella abortus equi* LPS (M. Freudenberg and H. Rau, unpublished results). This lower toxicity might possibly be due to the presence of a smaller extent and to the unusual nature (longer-chained) of the ester-linked fatty acids.

(1) J. Weckesser and H. Mayer. *FEMS Microbiol. Rev.*, **54** (1988) 143 – 154.
(2) A. P. Moran *et al. J. Bacteriol.*, **173** (1991) 618 – 626.

S19.2

Novel Lipophosphoglycan-Like Glycoconjugates Expressed on the Cell Surface of *Trichomonas vaginalis* and *Tritrichomonas foetus*

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Trichomoniasis, a common sexually transmitted infection, is caused by *Trichomonas vaginalis* in humans and by *Tritrichomonas foetus* in cattle, residing on the surface of the epithelium of the urogenital tract, where they derive most of their nutrients from the host. The parasites' means of attachment to the surface of the urogenital tract and avoidance of the host immune response is unknown. Partial characterization of the lipophosphoglycans (LPGs) has been reported (Singh, 1993); complete structural elucidation is underway, using a combination of chromatography, SDS-PAGE, enzymatic and chemical degradation, gas chromatography (GC), GC/mass spectrometry (MS), liquid secondary ionization tandem MS and matrix-assisted laser desorption ionization time-of-flight MS. The methodology and contributions of new mass spectrometric approaches utilized for the structural determinations will be highlighted. The presence of LPGs on the cell surface of the parasites was demonstrated through the galactose oxidase/NaB^3H_4 technique. The most striking and novel features of these LPG macromolecules are the presence of large amounts of GlcN in *T. vaginalis* and Fuc in *T. foetus* and of GalN in both parasites. Reductive radiomethylation of LPG from both parasites indicates that both the lipid-containing glycan core and the oligosaccharide portion have free amino groups as substituents. *T. vaginalis* binds strongly to the lectin ricin RCA-1 indicating the presence of terminal Gal, and *T. foetus* LPG binds (*ca.* 60%) to the lectin UEA-1 indicating the presence of terminal α1→2 linked Fuc. Treatment of the LPGs with PI-PLC liberated a ceramide lipid component. Nitrous acid deamination of LPGs resulted in the release of a phospholipid moiety containing *myo*-inositol. The LPGs appear to be anchored in the membrane *via* an Inos-P-(d18:0/16:0)ceramide. The presence of LPG-like glycoconjugates on the cell surface of trichomonad parasites may have an important role in host-parasite interactions.

S19.3

Structural and Immunochemical Characterization of *Haemophilus influenzae* Lipooligosaccharides

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Lipooligosaccharides (LOS) are the major glycolipids on the outer membrane of Gram-negative mucosal pathogens such as *Haemophilus* and *Neisseria* species. Unencapsulated strains of *Haemophilus influenzae,* which cause common respiratory tract infections, present their LOS directly to the host environment, whereas *H. influenzae* type b (Hib) strains, which cause meningitis, also produce a capsular polysaccharide which has been identified as a major virulence factor. In both unencapsulated and encapsulated *Haemophilus* strains, however, bacterial LOS also mediate the virulence of the pathogen.

The LOS of *H. influenzae* are structurally and antigenically diverse glycolipids whose surface-exposed epitopes suggest adaptive strategies used by the pathogen to evade the host's immune defense systems. *H. influenzae* can produce LOS epitopes which mimic host carbohydrate antigens and is also capable of rapidly varying the expression of some LOS epitopes. To study the relationships between LOS carbohydrate epitopes, phase variation and virulence, we have cloned an Hib gene cluster and generated a series of isogenic Hib LOS mutants by transposon mutagenesis.

O-Deacylated LOS samples from Hib strain A2 and two isogenic mutants were analyzed by electrospray mass