# LETTER TO THE EDITOR

## SOME CHARACTERISTICS OF A SMOOTH TYPE LIPOPOLYSACCHARIDE OF *CHLAMYDIA PSITTACI*

R. TOMAN[1], Ľ. ŠKULTÉTY[1], E. KOVÁČOVÁ[1], V. PÄTOPRSTÝ[2]

[1]Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava; [2]Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic

*Received December 17, 1996*

*Chlamydiae* are pathogenic, obligatory phagosomal intracellular parasites, which cause acute and chronic diseases in animals and humans (*1,2*). A lipopolysaccharide (LPS) and the 39.5 K major outer membrane protein are the major surface antigens of chlamydial cells (*3*). The former contains a trisaccharide of 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo), which represents an epitope shared by the whole genus (*4*). Further, it has been shown (*4*) that the chlamydial LPS is phenotypically of the rough (R) type. In two strains of *Chlamydia psittaci* and in *Chlamydia trachomatis* serotype $L_1$, the presence of smooth (S) LPS, in addition to the known R-LPS, has been reported (*5*). However, chemical composition and structure of this LPS could not be established. A glycolipid has been isolated from the supernatants of cultures infected with *C. trachomatis* (*6,7*). In that study, chromatographic and spectroscopic analyses of the glycolipid did not reveal the presence of either Kdo or heptose. It contained two fatty acids of C17 and C18:1, and from carbohydrates only gulose was detected.

This is the first report on the sugar composition of a chlamydial antigen sharing common properties with those of enterobacterial S-LPSs. A so far unknown S-LPS has been isolated from the supernatants of yolk sacs of embryonated chicken eggs infected with *C. psittaci* strain PK5082. The latter is associated with enzootic abortion in ewes.

*C. psittaci* strain PK5082 was grown in embryonated chicken eggs as described elsewhere (*8*). The elementary bodies were purified (*9*) and the supernatants were pooled, evaporated to a smaller volume, and tested for bacterial growth on glucose broth and on thioglycolate medium. Following these tests, which showed that the supernatant was negative for aerobic and anaerobic bacteria, ammonium sulphate was added to remove a large portion of protenious material from the supernatant. The precipitate was removed by centrifugation and the supernatant was dialyzed against tap water. The retentate was then partially evaporated, digested with bovine pancreatic trypsin (1:250, Serva) in phosphate-buffered saline, dialyzed, and finally lyophilized. The lyophilizate was extracted with hot phenol-water method (*10*) and the aqueous phase was fractionated on a column of Octyl-Sepharose CL-4B (Pharmacia). The polymer material eluted with 50% aqueous ethanol was resistant to heat and proteolytic digestion, and gave a ladder-like banding pattern in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. On depolymerization with acid, mainly glucose (35.6%), rhamnose (28.9%), galactose (20.8%) together with small amounts of mannose (2.9%) and fucose (1.8%) (all in mole %) were detected as the corresponding alditol acetates and trimethylsilyl methyl glycosides by gas chromatography-mass spectrometry. L-Glycero-D-manno-heptose and Kdo were also detected in the amounts of 4.7 and 3.1%, respectively. The sugars present in amounts less than 1% are not given here. The S-LPS isolated gave a positive reaction with polyclonal rabbit serum against *C. psittaci* PK5082 in immunoblot analysis and enzyme-linked immunosorbent assay. With the latter method, no positivity was observed when the S-LPSs from *Salmonella typhimurium* and *Coxiella burnetii* were tested.

**Abbreviations:** LPS = lipopolysaccharide; Kdo = 3-deoxy-D-manno-oct-2-ulosonic acid; R = rough; S = smooth

In conclusion, these results indicate that the mechanism of LPS variation may take place also in *Chlamydiae*, which have been reported *(9,11,12)* to lack the O-chains in their LPSs. A release of soluble S-LPS antigen could play a role in the immunopathology associated with diseases caused by *Chlamydiae*.

**Acknowledgement.** We thank Mrs. J. Dobiášová for skilful technical assistance.

## References

*1.* Schachter J, Caldwell HD, *Annu. Rev. Microbiol.* **34**, 285, 1980.
*2.* Moulder JW, *Microbiol. Rev.* **55**, 143, 1991.
*3.* Schachter J, *Curr. Top. Microbiol. Immunol.* **138**, 109, 1988.
*4.* Brade H, Brade L, Nano FE, *Proc. Natl. Acad. Sci. USA*, **84**, 2508, 1987.
*5.* Lukáčová M, Baumann M, Brade L, Mamat U, Brade H, *Infect. Immun.* **62**, 2270, 1994.
*6.* Stuart ES, Tirrell SM, MacDonald AB, *Immunology* **61**, 527, 1987.
*7.* Stuart ES, MacDonald AB, *Immunology* **68**, 469, 1989.
*8.* Sádecký E, Trávniček M, Balaščák J, Brezina R, Kazár J, Úrvölgyi J, Flešár I, Čižmár J, *Vet. Med.* (Prague) **23**, 25, 1978.
*9.* Brade L, Schramek Š, Schade U, Brade H, *Infect. Immun.* **54**, 568, 1986.
*10.* Westphal O, Jann K, *Methods Carbohydr. Chem.* **5**, 83, 1965.
*11.* Nurminen M, Rietschel ET, Brade H, *Infect. Immun.* **48**, 573, 1985.
*12.* Toman R, Leinonen M, Saikku P, In Mardh PA, La Placa M, Ward M (Eds): *Proceedings of the European Society for Chlamydia Research.* University of Uppsala, Uppsala, p. 14, 1992.