SEROLOGICAL CROSS-REACTIONS OF LIPID A COMPONENTS OF LIPOPOLYSACCHARIDES ISOLATED FROM *CHLAMYDIA PSITTACI* AND *COXIELLA BURNETII*

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The presence of a lipid-carbohydrate-protein complex in the cell wall of chlamydiae (*1*), the content of 2-keto-3-deoxyoctonic acid in the chlamydial group-antigen (*2*), and the demonstration of an endotoxin-like activity in chlamydiae (*3*) suggest the possible existence of an endotoxic lipopolysaccharide (LPS) in the chlamydial cell wall as it is known in rickettsiae (*4, 5*). We isolated LPS from chlamydiae and compared it with rickettsial LPS.

Chlamydia psittaci strain 5082 of enzootic abortion of ewes and *Coxiella burnetii* strain Nine Mile in phase II grown in chick embryo yolk sacs were killed by formaldehyde and purified as described (*5*). The purified dried rickettsial and chlamydial cells were extracted by the phenol-chloroform-petroleum ether method (*6*) modified as follows. The cells were homogenized (Potter glass homogenizer, 3 min) and sonicated (18 kHz, 250 W, 3 min) with extraction mixture. The treated cells were centrifuged off (5,000 × g, 15 min) and the supernatant was collected. The procedure was repeated twice more. Petroleum ether and chloroform were removed from the pooled supernatants by evaporation in vacuo. Acetone was then added to the phenol until a precipitate was formed. The latter was washed three times by acetone, then by ether, dried and dissolved in water. Undissolved substances were removed by low speed centrifugation and the supernatants were lyophilized. The isolated white fluffy substances (presumably LPS) were analysed by the phenol-sulphuric acid method with glucose as a standard for the presence of neutral sugar. The content of neutral sugars in such crude preparations (related to the starting material) was 9.7% in *C. burnetii* and 8.6% in *C. psittaci*. To obtain lipids A, the isolated substances were subjected to mild acid hydrolysis (1% acetic acid, 100 °C, 60 min). The precipitates formed were treated with 0.25 M NaOH (56 °C, 30 min) and used for sensitization of sheep erythrocytes for the passive haemolysis test (*7*). Anti-lipid A sera were prepared by immunization of rabbits with purified cells of *C. burnetii* or *C. psittaci* that had been subjected to mild acid hydrolysis (1% acetic acid, 100 °C, 2 hr). The immunization schedule was as follows: 100, 200, 300 and 500 μg on days 1, 4, 7 and 11, respectively; bleeding on day 17. In the passive haemolysis test, both sera reacted to the same titres (*C. burnetii* anti-lipid A serum titre 512; *C. psittaci* anti-lipid A serum titre 256) with erythrocytes sensitized either with lipid A of *C. burnetii*, or with the alkali-treated precipitate obtained following mild acid hydrolysis of the substance isolated from *C. psittaci*.

The serological cross-reactions obtained indicate the presence in *C. psittaci* of lipid A whose chemical structure is basically similar to that of lipid A of *C. burnetii*. This fact together with the finding of neutral sugars offers evidence for the existence of LPS also in the chlamydial cell wall. As follows from our preliminary experiments on actinomycin D-sensitized mice, toxicity of chlamydial LPS is low like that of *C. burnetii* LPS preparations.

References

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