SHORT COMMUNICATION

Demonstration of Chlamydial Endotoxin-like Activity

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A *Chlamydia psittaci* strain grown on McCoy cell monolayers showed endotoxin-like activity by both the limulus amoebocyte lysate assay and the rabbit pyrogen test.

INTRODUCTION

The lipid-carbohydrate-protein complexes of chlamydial walls are biochemically similar to the endotoxic walls of Gram-negative bacteria (Manire & Tamura, 1967). Furthermore, Dhir *et al.* (1972) showed that chlamydial group antigen contains 2-keto-3-deoxyoctonic acid which is almost identical to the ketodeoxyoctonic acid from salmonella lipopolysaccharide. Despite such observations, however, endotoxin has not been demonstrated in chlamydiae. The work described here was undertaken to determine whether endotoxin-like activity could be demonstrated for these organisms.

METHODS

Organism. *Chlamydia psittaci* strain RA644 was isolated from a lesion in a parrot by intracerebral inoculation of an infected sample into mice. The identity of the organism was confirmed by the presence in impression slides, stained by the technique of Gimenez (1964), of characteristic intracytoplasmic inclusions, elementary bodies and their earlier developmental forms, as well as by the demonstration of chlamydial-specific antigens using both the complement-fixation test and the enzyme-linked immunosorbent assay (Lewis *et al.*, 1977). Before use the organism was passaged once in 5-iodo-2-deoxyuridine (IUDR)-treated McCoy cell culture (Wentworth & Alexander, 1974).

Culture and harvesting of chlamydiae. To obtain sufficient organisms for testing, each of 20 cell culture bottles (946 ml) was seeded with 3×10^6 McCoy cells in 60 ml of endotoxin-free Eagle medium (Microbiological Associates, Walkersville, Md., U.S.A.) containing 10% (v/v) foetal bovine serum, 2 mM-glutamine, 30 mM-glucose and $25 \mu\text{g}$ IUDR ml^{-1} . The serum contained no endotoxin detectable by the limulus amoebocyte lysate (LAL) assay (Levin & Bang, 1964). The culture bottles and all other glassware were rendered pyrogen-free before use by exposure to 180°C for 4 h. After incubation of the cultures for 3 d at 37°C , the medium was removed and 10 of the monolayers were each inoculated with 10^6 mouse intracerebral mean lethal doses (Reed & Muench, 1938) of *C. psittaci* suspended in endotoxin-free Eagle medium containing glucose and glutamine as before but with only 2% (v/v) foetal bovine serum and no IUDR. This medium alone was added to the other 10 monolayers, and incubation of all monolayers was continued. Daily, a few cells were scraped from the cultures, stained (Gimenez, 1964) and examined microscopically with respect to numbers of chlamydiae and purity of the cultures. When the stained cells showed that monolayers were highly infected, the organisms were collected; the cells were disrupted in their medium by three freeze/thaw cycles, debris was removed by centrifuging at $500 g$ for 15 min, and the chlamydiae were pelleted from the pooled supernatant fluid by centrifuging at $22\,700 g$ for 1 h. The pellet was then suspended in 20 ml pyrogen-free distilled water and tested for sterility both aerobically and anaerobically. The 10 uninfected control monolayers were processed in the same way as the infected monolayers.

Assay for endotoxin. For the LAL assay (Levin & Bang, 1964), dilutions of the suspensions from both the infected monolayers and the uninfected controls were prepared in pyrogen-free distilled water and 0.2 ml samples were added to vials containing LAL (Microbiological Associates) standardized to detect as little as 0.20 ng endotoxin ml⁻¹. Where positive reactions occurred, a solid gel formed during incubation at 37 °C for 1 h. A positive control of 0.20 ng *Escherichia coli* O111:B4 endotoxin (Microbiological Associates) and a negative control of pyrogen-free distilled water were included in the test system.

The suspensions from both the infected and uninfected control monolayers were also assayed for endotoxin by the rabbit pyrogen test; this was done as described by Miller (1970) except that 1.5 ml were inoculated rather than 10 ml.

RESULTS AND DISCUSSION

Three days after chlamydial inoculation of the monolayers, microscopic examination of cells scraped from them and stained revealed numerous chlamydia.

The end-point dilution for endotoxin by the LAL assay was 1:32768 and 1:8 for the infected and uninfected cultures, respectively. In the rabbit pyrogen test, the corresponding positive end-point dilutions obtained were 1:64 and < 1:4. The rabbit pyrogen test is reportedly much less sensitive than the LAL assay for endotoxin (Cooper *et al.*, 1971).

The endotoxin-like activity of the chlamydial suspension was not diminished by keeping it in a water bath at 100 °C for 1 h before testing. The relation of this heat-stable activity to the extremely heat-labile toxic factor long recognized as associated with chlamydiae (Rake & Jones, 1944) remains to be defined.

Chlamydiae resemble Gram-negative bacteria in several characteristics but differ from them in others (Moulder, 1964; Costerton *et al.*, 1975, 1976). The results of this study indicate that one of the shared properties may be the presence of endotoxin. Because relatively large amounts of chlamydiae can now be grown readily in cell culture, it should be feasible to isolate and characterize chemically the moiety producing a positive reaction in both the LAL and rabbit pyrogen test and thus determine conclusively whether it is indeed an endotoxin.

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