Elementary Body Envelopes from *Chlamydia psittaci* Can Induce Immediate Cytotoxicity in Resident Mouse Macrophages and L-Cells

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Isolated, purified *Chlamydia psittaci* elementary body envelopes at a high multiplicity of infection (1,000:1) are capable of inducing immediate cytotoxicity in resident mouse macrophages and 929 L-cells.

In 1971 Kordova and Wilt and Kordova et al. (3, 4) described a cytotoxic reaction in L-cells and in macrophages that was a result of ingestion of large numbers of infectious *Chlamydia psittaci* 6BC. This cytotoxic event occurred early in the developmental cycle (3 to 6 h post-inoculation) in contrast to the late damage which occurred in the host cell as a result of natural chlamydial infection. Kordova et al. (4), and subsequently Todd and Storz (11), showed evidence for the release of lysosomal enzymes late in the chlamydial developmental cycle and postulated that hydrolytic activity provided cell lysis and the means of escape for mature elementary body (EB) progeny. Early cytotoxicity was dependent on ingestion of a high multiplicity of infection (MOI) rather than on subsequent replication. In the L-cell there was disruption of neutral red-staining granules and an early leak of acid phosphatase. An explanation for early cytotoxicity was that large numbers of EB simulate the late stages of the developmental cycle and trigger early lysosomal enzyme release and, hence, rapid host cell cytopathic effects (4).

A few years later, the phenomenon was examined in depth by Moulder et al. (6), using primarily the *C. psittaci* 6BC-L-cell system. With an inoculum of 500 tissue culture 50% infectious doses, monolayer formation was impaired and cells rounded and began to die. The rapidity with which toxic effects occurred led the authors to refer to the phenomenon as “immediate cytotoxicity.” Induction of cytotoxicity occurred when UV light-treated EB were used; however, lysosomal hydrolases were not present as measured by acid phosphatase. The Moulder group thus hypothesized that each act of parasite-mediated ingestion produced an independent lesion in the L-cell cytoplasmic membrane; the L-cell was unable to repair the many lesions produced, and cell death ensued.

Taverne et al. (10) reported similar findings for *Chlamydia trachomatis* organisms in mouse macrophages and BHK cells. Furthermore, macrophages containing a cytotoxic dose of chlamydiae, injected intravenously into syngeneic mice, induced severe shock in 45% of the mice within 1 h. Similarly, cytotoxic macrophage lysates induced measurable erythematous lesions in the skin of guinea pigs, whereas disrupted macrophages from uninoculated control animals produced no hyperemic lesions after intradermal injection (9). It was speculated that the cellular events of early cytotoxicity could be the in vitro equivalent of (i) toxic death in mice produced by intravenous injection of large doses of EB described by Rake and Jones (7) and (ii) the severe epithelial cell damage seen in the eyes of trachoma patients (9). Wyrick et al. (12), Gardner (M. Gardner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, D6, p. 70), and Kuo (5) added information on immediate cytotoxicity in macrophages by using *C. psittaci* and both *C. trachomatis* and lymphogranuloma venereum biopars, respectively. Unlike that observed in the non-professional phagocytic L-cells, cytotoxic damage to mouse macrophages required only 10 50% infectious doses of *C. psittaci*, 30 infectious units of *C. trachomatis* serotype B, and 100 infectious units of lymphogranuloma venereum 434. Reticulate bodies were not infectious for L-cells but were internalized by macrophages. Reticulate bodies at an MOI of up to 1,000:1 were incapable of causing immediate cytotoxicity in thioglycolate-elicited macrophages (1). The reactions appeared specific for EB since polystyrene latex spheres, up to an MOI of 1,000:1, also did not induce immediate cytotoxicity (12).

In the present study 24-h-old mouse peritoneal macrophages were inoculated with isolated, purified EB envelopes and examined for an immediate cytotoxic reaction. The EB envelopes, derived from *C. psittaci* Cal 10, were obtained by Mickle disruption of whole EB and subsequent purification on a linear sucrose gradient (2). The purified EB envelope preparations were examined by electron microscopy of shadow cast preparations and stained thin sections as well as by scanning 24-h-stained L-cell monolayers inoculated with envelope counts of 500:1, 200:1, and 100:1. EB contamination was judged to be less than 1%. Fig. 1 is representative of the intactness of the envelope sacculus as well as the retention of the surface projections on the purified EB envelopes. The concentration of envelopes was measured from a standard curve of turbidity (optical density) versus total particle count as measured by electron microscopy (8). The envelopes, suspended in phosphate-buffered saline or tissue culture medium 199 devoid of the phenol red indicator to various MOI, were centrifuged at 733 × g for 15 min onto either resident or thioglycolate-elicited macrophage monolayers (2 × 10⁶ macrophages per petri dish [10 by 35 mm]). The infected monolayers were reincubated at 37°C in an atmosphere of 5% CO₂ at timed intervals, fractions of macrophage culture supernatant were monitored for the release of the soluble cytoplasmic enzyme lactic dehydrogenase (LDH) (12). The positive control consisted of an uninfected macrophage monolayer treated at room temperature for 5 min with 0.003% Triton X-100; an average of 0.073 U of LDH were released.

The initial experiments were performed with thioglycolate-elicited macrophages, since our previous studies had
indicated no difference in whole EB-induced immediate cytotoxicity results between resident and elicited macrophages. However, no immediate cytotoxicity could be demonstrated in the elicited macrophages inoculated with purified EB envelopes at an MOI of 1,000:1. Lengthening the time of incubation from 6 h to 14 and 24 h did not produce an LDH release greater than that in the uninfected control cells. In contrast, when resident macrophages were employed, purified EB envelopes at an MOI of 1,000:1 did induce immediate cytotoxicity at a rate comparable to intact EB at an MOI of 100:1 (Fig. 2). With a lower EB envelope inoculum (MOI, 100:1), the amount of LDH released was similar to control macrophages. EB envelopes at the high MOI (1,000:1) also induced immediate cytotoxicity in 929 L-cell monolayers as determined by the rounding of the L-cells and their detachment from the monolayer within 1 to 2 h post-inoculation; LDH release was low in L-cells and could not be used as a reproducible parameter of cytotoxicity.

One set of conflicting data which we cannot explain involves the loss of EB-induced cytotoxicity on heating the EB (56°C for 15 min), whereas isolated EB envelopes heated in the same manner still retained the capacity to induce toxicity in resident macrophages (12).

Large batches of EB, of differing infectivity titers, are pooled to generate sufficient quantities of envelopes. In general, the infectivity of any one batch is 50% or less, which means that significant numbers of "noninfectious" organisms are also present from which envelopes are obtained. The average uptake of EB envelopes, as measured by radioactivity in resident macrophages, is 37%, and in L-cells it is 43% (2); but it is difficult to translate this information into the actual number of infectious envelopes internalized. Thus, we can only conclude that large numbers of isolated EB envelopes are capable of inducing immediate cytotoxicity in target host cells.

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