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Effect of Seminal Plasma on *Chlamydia trachomatis* Strain LB-1 in Cell Culture

LAVELLE HANNA,[1]* HERMINE KESHISHYAN,[1] GEORGE F. BROOKS,[2] DANIEL P. STITES,[2] AND ERNEST JAWETZ[1]

Departments of Microbiology and Immunology[1] and Medicine and Laboratory Medicine[2], University of California, San Francisco, California 94143

Human seminal plasma inhibited formation of strain LB-1 chlamydial inclusions in McCoy cells proportional to the concentration of seminal plasma added after chlamydial adsorption.

Chlamydia are among the most common sexually transmitted organisms and are probably responsible for at least one-half of the cases of nongonococcal urethritis in males. We questioned whether genital secretions might influence the infectivity of such agents (6) or the susceptibility to infection of the host cells. We describe here the effect of seminal plasma (SP) on standardized infections of *Chlamydia trachomatis* strain LB-1 in cultured cells in vitro and a few parameters which determined inclusion formation in such cells.

Cell cultures. McCoy cells were grown on cover slips in Leighton tubes in Eagle minimum essential medium containing 10% fetal calf serum plus gentamicin (50 μg/ml) and fungizone (2.5 μg/ml). The maintenance medium (MS) contained gentamicin with 2% glucose plus cycloheximide (1 μg/ml). One-milliliter volumes of cell suspensions containing 3×10^5 to 3.5×10^5 cells were added to Leighton tubes and incubated at 35°C.

Cover slips were removed after 50 to 55 h of incubation at 35°C, fixed in absolute methanol, and stained with Giemsa stain. Triplicate preparations were examined, and inclusions in 30 random fields per cover slip (40 to 50 cells per field) were counted by two observers with a 40× oil immersion objective (final magnification, 480×) (2). Counts of inclusions formed in the presence of SP were expressed as a percentage of the number of inclusions in control monolayers in the absence of SP in the same experiment.

Chlamydial inoculum. *C. trachomatis* strain LB-1 (immunotype L2) was diluted in MS to produce 80 to 120 inclusion-forming units per 30 microscopic fields, with about 10% of the cells being infected.

SP. Human SP was collected from normal donors, pooled, centrifuged at 300 × *g* for 10 min to remove sperm, filtered (0.45-μm pore size; Millipore Corp.), and stored frozen at −20°C in aliquots until tested (7). Dilutions were made in minimum essential medium. No antichlamydial antibodies were detected in the pooled SP by indirect microimmunofluorescence employing anti-human whole serum globulin (1).

Procedure A. Medium was removed from cell monolayers, and 1 ml of LB-1 dilution was added and adsorbed for 3 h at 35°C. Inoculum was removed, cells were washed once with Hanks balanced salt solution (BSS), and 1 ml of MS with or without diluted SP was added. Tubes were incubated at 35°C for 3 h. Since high concentrations of SP were toxic if left on the cells, fluid was removed, the monolayer was again washed once with BSS, and fresh MS was added. Monolayers were then incubated at 35°C for a total of 50 to 55 h.

Procedure B. Medium was removed from cell monolayers, 0.1 ml (volume sufficient to cover the cell sheet) of diluted SP was added, and monolayers were incubated at 35°C. After 30 min, 1 ml of diluted LB-1 was added and adsorbed at 35°C for 3 h. The SP and LB-1 were removed, cells were washed once with BSS, 1 ml of fresh MS was added, and monolayers were incubated at 35°C.

Procedure C. Medium was removed from cell monolayers. Equal volumes of diluted LB-1 and diluted SP were inoculated simultaneously and allowed to interact for 3 h at 35°C. The inoculum was removed, the monolayers washed once with BSS, fresh MS was added, and the monolayers again were incubated at 35°C.

Procedure D. Medium was removed from cell monolayers, and 1 ml of diluted LB-1 was added and adsorbed for 3 h at 35°C. Inoculum was removed, cells were washed once with BSS, 1 ml of MS was added, and cells were incubated at 35°C for 3 h. MS was removed, and fresh MS with or without diluted SP was added for 3 h at 35°C. The fluid was removed, fresh MS was added, and monolayers were incubated at 35°C.

Procedure E. Equal volumes of diluted SP

and diluted LB-1 were combined. After 30 min at room temperature, monolayers were inoculated with 1-ml volumes of these mixtures and adsorbed at 35°C for 3 h. The inoculum was removed, the monolayers were washed once with BSS, fresh MS was added, and tubes were incubated at 35°C.

The inhibition by SP of the formation of chlamydial inclusions in cell cultures depended on three distinct factors. With infection yielding over 250 inclusions per 30 fields, SP diluted 1:10 reduced the number of inclusions by only 30%. Conversely, with infection yielding only 10 inclusions per 30 fields, SP totally inhibited formation of inclusions. We therefore used an inoculum yielding 80 to 120 inclusions per 30 fields.

SP diluted 1:5 was always damaging for McCoy cells. If left on the cells for the entire incubation period of 50 to 55 h, dilutions of 1:10 and 1:25 produced morphological damage to cells seen microscopically. Dilutions of 1:10 or greater, removed after 3 h of contact with the cells, resulted in no microscopically visible cell damage or loss of cells.

The sequence in which the chlamydial inoculum and the SP were added clearly determined the extent of inhibition. In repeated tests, procedure A with SP diluted 1:10 resulted in reduction of the number of inclusions. However, no inhibition by SP could be detected when the SP was added 30 min before the LB-1 and left on for the 3-h absorption period (procedure B), when LB-1 and SP were added at the same time (procedure C), or when the SP was added 3 h after removal of the LB-1 inoculum and left on for 3 h (procedure D). Likewise, mixing LB-1 and SP and incubating the mixture for 30 min before adding it to cells (procedure E) had no effect.

The results described above were obtained by using SP diluted 1:10. Figure 1 shows the percentage of inclusions formed after interactions of increasing dilutions of SP with chlamydia in our system. Percentages shown represent averages of five replicate experiments. The range of results for each dilution is also given. Examples of actual inclusion counts are given in Table 1. Inclusions which formed were entirely normal in appearance, regardless of the concentration of SP to which the cells had been exposed.

During the passage of sexually transmitted organisms from one host to the next, the organisms are suspended in secretions of the genital tract for some time. If such secretions were inimical to the viability of the organisms, this might reduce the efficiency of passage and provide some degree of protection for the recipient host. Chlamydia are very common sexually transmitted organisms, and nongonococcal urethritis is a frequent clinical manifestation. It is reasonable, therefore, to inquire whether SP affects the infectivity of chlamydia. We used strain LB-1 (immunotype L-2), an isolate from the genital tract. There is no available method to test this in vivo, but our laboratory has found such effects in vitro. We reported that lysozyme (a constituent of SP, cervical secretions, and tears) somewhat reduced the infectivity of chlamydia for cultured cells (3), and this has been confirmed (4). We have also reported inhibition of chlamydia by interferon, another nonspecific inhibitor (2).

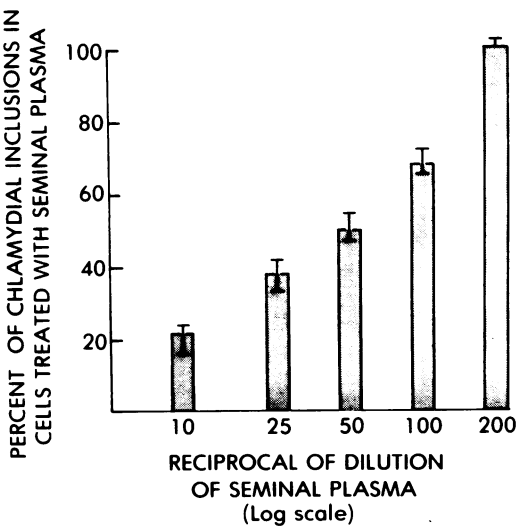

FIG. 1. *Inhibition of chlamydial inclusion formation by SP expressed as a percentage of the number of inclusions in parallel control preparations (range of results from five replicate experiments is shown at top of each bar).*

TABLE 1. *Inclusion counts formed in the presence of SP*

SP dilution (0.1 ml)	Inclusion counts per 30 fields[a]		
		Avg	%
0	123, 120, 125	123	100
1/10	30, 28, 32	30	24
1/25	54, 52, 51	52	42
1/50	65, 68, 70	68	55
1/100	92, 86, 89	89	72

[a] Triplicate cover slips.

The results presented here demonstrate unequivocally that (i) SP in dilutions of 1:10 to 1:100 inhibits the formation of LB-1 inclusions in McCoy cells; (ii) within this concentration range, SP is inhibitory in proportion to concentration; (iii) SP does not inactivate infective chlamydia in 30 min of contact; (iv) SP is toxic to cultured cells unless diluted 1:10 or more. The inhibitory

effect of SP was evident only if it acted on cells which had either adsorbed or perhaps ingested chlamydia. Under all other conditions tested, no inhibition was observed. The results suggest an effect on cells, but its nature is unexplained. The SP pool contained no antibody to any of 11 *C. trachomatis* antigens demonstrable by indirect immunofluorescence (1), the most sensitive method available, and had no direct effect on inclusion formation either when preincubated or when added simultaneously to the cell monolayers, indicating that the effect was not antibody mediated.

After this work was completed, Mardh et al. (4) also reported that SP and some of its constituents could interfere with the ability of chlamydia to form inclusions in cultured cells. The similar results in his study (with *C. trachomatis* immunotype F) and our study (with immunotype L-2) suggest that inhibition by SP may apply to many *C. trachomatis* strains. In our studies, we further explored the role of sequential events in the test system. The observed antichlamydial effect may be equivalent to the antibacterial action demonstrated in the past (5, 6).

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