Differential Susceptibility of Chlamydiae to Exogenous Fibroblast Interferon

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Mouse fibroblasts (L cells) were incubated for 5 h with 1,000 U of murine fibroblast interferon (MuIFN α+ β) per ml and then were infected with either Chlamydia trachomatis (LGV 440), C. psittaci (6BC), or C. psittaci (Cal 10). Intracellular development of C. trachomatis was reduced 90% in interferon-treated cells 24 h after infection when compared with controls, whereas C. psittaci growth was not affected in interferon-treated cells.

Chlamydiae are obligate intracellular procaryotic pathogens ubiquitously distributed in nature. The genus comprises two species: C. psittaci and C. trachomatis. The intracellular replication of both species is similar and involves the orderly alternation of two morphologically and biochemically distinguishable cell forms. The elementary body is the infectious form of the organism, but it is incapable of intracellular replication, whereas the reticulate body is the replicative form, incapable of initiating the infectious cycle (12). This cycle of development is unique to chlamydiae and is the principal reason that the two species are classified together in a single genus (11). The two species also share a single genus-specific antigen (3), but they are otherwise antigenically unrelated (2). The species exhibit essentially no DNA homology (10) and may also be distinguished based on their glycogen-producing capacity and their differential susceptibility to sulfa drugs (12).

Chlamydiae are known to induce interferon production (5, 8) and are inhibited in the presence of exogenous interferon (4, 9), but each of these studies reported on the effects of interferon with C. trachomatis strains. We report here that representatives of C. trachomatis and C. psittaci may also be separated based on their differential susceptibility to replication in host cells that were first incubated in the presence of murine fibroblast-derived interferon (muIFN α+ β).

Interferon was provided by Edward A. Havell of The Trudeau Institute, Saranac Lake, N.Y. The material was produced by polyI:polyC-induced mouse fibroblasts (Lpa strain) and titrated by using L cells and vesicular stomatitis virus as the indicator, as described by Havell and Vilcek (7). C. trachomatis (LGV 440) was propagated in HeLa cells, and C. psittaci (6BC and Cal 10) was propagated in L cells. Chlamydiae were harvested from infected cells by sonication and differential centrifugation procedures that have been described in detail elsewhere (1). Infectious titers were determined by the 50% infective dose method, best described by Hatch (6). L cells were plated onto 15-mm-diameter round cover slips in 35-mm-diameter tissue culture dishes at a density of 2 × 105 cells per cover slip. Cells were allowed to attach and spread for 2 h at 37°C in an atmosphere composed of 5% CO2 in air in medium 199 containing 0.1% sodium bicarbonate, 2 mM L-glutamine, 10% heat-inactivated fetal calf serum (Flow Laboratories, Inc., Rockville, Md.), and 10 μg of gentamicin sulfate per ml (growth medium). The medium was then aspirated, fresh medium or fresh medium containing 1,000 U of interferon (IFN) per ml was added to the dishes, and the cells were incubated for 5 h as before. Monolayers were then washed twice with phosphate-buffered saline and infected with a dilution of either C. trachomatis (LGV 440), C. psittaci (6BC), or C. psittaci (Cal 10). Chlamydial dilutions were adjusted so that approximately 50% of the cells would be infected. There was a degree of variability observed, and infected control populations ranged from 11 to 44% infected. After adsorption of the inoculum for 1 h at 37°C, fresh growth medium was added, and the cells were reincubated for an additional 22 to 24 h to allow for cytoplasmic inclusion development, a microscopically visible indicator of chlamydial replication. After incubation, cover slips were fixed in absolute methanol and stained with Giemsa, and the percentage of inclusion-bearing cells was quantitated by light microscopy. The data in
TABLE 1. Effect of mouse fibroblast interferon on the growth of various chlamydial strains in L cells

<table>
<thead>
<tr>
<th>Chlamydial strain tested</th>
<th>% of inclusion-bearing cells&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Inhibition&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. trachomatis (LGV 440)</td>
<td>1 ± 1</td>
<td>91</td>
</tr>
<tr>
<td>C. psittaci (6BC)</td>
<td>34 ± 7</td>
<td>17</td>
</tr>
<tr>
<td>C. psittaci (Cal 10)</td>
<td>39 ± 3</td>
<td>10</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean of triplicate samples ± standard deviation.

<sup>b</sup> Calculated by: [(percent control cells — percent IFN-treated cells) / (percent control cells)] × 100.

Table 1 compare the inclusion-bearing fraction of cells in control and IFN-treated L cells. There was a greater than 90% reduction in the percentage of inclusion-bearing cells for IFN-treated L cells infected with C. trachomatis (LGV 440) as compared with untreated control LGV 440-infected L cells. Very little inhibition of inclusion development was observed in the C. psittaci-infected, IFN-treated cells as compared with controls.

These data provide evidence for a possible distinction between the two species of Chlamydia. It may be of significance that earlier reports (4, 5, 8, 9) concerning the effects of exogenous interferon on chlamydial development were done with C. trachomatis strains. Our results are in agreement with previous studies in that LGV 440 did not form inclusions in IFN-treated L cells, but despite this control of the potency of IFN used, neither strain of C. psittaci was affected. Additional strains should be tested before definitive conclusions are made concerning differences in species susceptibility, but the information provided here shows that the effects of IFN treatment may vary between selected chlamydial strains.

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We thank Edward A. Havell for the generous gift of interferon.

ADDITION IN PROOF

Replication of C. psittaci strains was not affected when L cells were incubated in medium containing 10<sup>4</sup> U of interferon per ml, 10 times the dose required to reduce C. trachomatis replication by greater than 90%.

LITERATURE CITED