Growth of *Chlamydia trachomatis* in McCoy Cells Treated with Cytochalasin B

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When the fungal metabolite cytochalasin B was added to McCoy cells, multinucleated giant cells developed. Monolayers of these cells proved as efficient as irradiated cells for the growth of three different serotypes of *Chlamydia trachomatis* and for the primary isolation of chlamydiae from clinical specimens obtained from patients attending a venereal disease clinic. Cytochalasin treatment of McCoy cells provides a convenient alternative to irradiation and should be of value in the isolation of chlamydiae from the eye and genital tract.

Both ocular and genital strains of *Chlamydia trachomatis* were first grown in the yolk sac of embryonated eggs (7, 11). Recently, however, cell culture techniques have been developed for the growth of these organisms. To date BHK cells (3), HeLa 229 cultures pretreated with diethylaminoethyl-dextran (9), normal McCoy cells (6), and irradiated McCoy cells (5) have been used for the primary isolation of *C. trachomatis*. In this laboratory irradiated McCoy cells have proved better for the growth of chlamydiae than normal McCoy, Bristol HeLa, or BHK cells despite the additional complication of irradiation.

Cytochalasin B (Cyt B), a metabolite of a mold *Helminthosporium dematioides* (1), induces formation of multinucleated giant cells by preventing cytoplasmic cleavage after nuclear division (8). The present study shows that McCoy cells treated with Cyt B are as effective as, and more convenient than, irradiated cells for growing chlamydiae.

**MATERIALS AND METHODS**

Organisms. Three strains of *Chlamydia trachomatis* were used in this study. (i) A genital trachoma-inclusion conjunctivitis (TRIC) agent, T181, was isolated in this laboratory in irradiated McCoy cell cultures from the urethra of a man suffering from nongonococcal urethritis. This isolate has been serotyped as type E by J. Treharne at the Institute of Ophthalmology, London WC1, U.K., using the micro-immunofluorescent serotyping method (12). A pool of this isolate made from the seventh irradiated McCoy cell passage and stored with 10% sorbitol (10) at −70°C was used in this study. The other strains used were: (ii) strain HAR-1, serotype A, and (iii) strain 434, serotype lymphogranuloma venereum (LGV) II.

The latter two strains were supplied as 50% yolk-sac suspensions by J. Schacter, Francis I. Proctor Foundation, University of California, San Francisco, Calif. In this laboratory they were passed into irradiated McCoy cell cultures from which pools were made as with (i).

Clinical specimens. Fifty-one specimens were collected from 41 patients attending a venereal disease clinic. Urethral swabs from males or cervical swabs from females were placed in sorbitol transport medium (10), snap frozen at −70°C, and stored for 1 to 6 weeks before testing.

Cyt B. Cyt B (manufactured by Imperial Chemical Industries Ltd., Cheshire, England; distributed by Aldrich Chemical Co., Inc., Milwaukee, Wisc.) was dissolved in dimethyl sulfoxide at a concentration of 1 mg/ml, dispensed in 0.1-ml portions, and stored at −20°C. Appropriate dilutions of Cyt B in McCoy growth and maintenance media (10) were made from this stock. A fresh vial was retrieved each time Cyt B was used, and dilutions were made immediately before addition to the cells. In control experiments, analogous concentrations of dimethyl sulfoxide added to the media had no demonstrable influence on the growth of McCoy cells or the development of chlamydiae.

Cell culture tubes. Plastic flat-bottomed vials (E 10/CSC/B, Turner-Stayne Laboratories Ltd., Bishop Auckland, Co. Durham, U.K.) containing a 12-mm diameter cover slip were used in all the experiments.

Irradiated McCoy cell cultures. Preparation of irradiated McCoy cell cultures has been described (10). Briefly, 1.5 × 10⁶ cells irradiated with 5,000 rads from an X-ray source 4 to 10 days previously were seeded in 1 ml of growth medium into the plastic tubes. They were incubated at 35°C and used for inoculation 1 to 3 days later.

Cyt B-treated McCoy cell cultures. Preliminary experiments (see Results) were carried out with different concentrations of Cyt B (0.3, 1, 3, and 10 μg/ml) added to McCoy cell monolayers before inoculation and/or at the time of inoculation to determine the effect of Cyt B on the morphology of the McCoy cells and on the growth of T181. For subsequent experiments McCoy cell cultures containing 10⁵ cells/tube...
in 1 ml of growth medium were treated with 1 μg of Cyt B per ml for 72 h before inoculation. The Cyt B was added either to newly formed monolayers or to the cell suspension before the cells were seeded into the culture tubes. Prior to inoculation the growth medium was replaced with 1 ml of maintenance medium containing 1 μg of Cyt B per ml.

**Normal McCoy cell cultures.** These were made at the same time as the Cyt B-treated cell cultures. Tubes were seeded with 10⁴ cells/tube, grown 3 to 4 days in ununsupplemented growth medium, and changed to maintenance medium immediately before inoculation.

**Growth of chlamydiae from pools.** Normal, Cyt B-treated and irradiated McCoy cell cultures were each inoculated with 0.25 ml of appropriate dilutions of the chlamydia pools, centrifuged for 1 h in an MSE Super Medium centrifuge at about 2,500 × g, and incubated at 35 C. T181 and HAR-1 were incubated for 48 to 72 h and the LGV strain for 50 to 48 h. Cover slips were then fixed in methanol and stained with Giemsa, and the total number of inclusions per cover slip was counted by dark-field microscopy, from which the number of inclusion-forming units per milliliter was estimated. For irradiated and Cyt B-treated cells, only dilutions giving 60 to 1,000 inclusions were used; analogous dilutions were also used for normal McCoy cell cultures, though the number of inclusions was often <60.

**Isolation of chlamydiae from clinical specimens.** This procedure has been described previously (10). Each specimen was inoculated into two Cyt B-treated and two irradiated McCoy cell cultures, 0.25 ml/tube. Cyt B cultures were coded and the cover slips were read blind, so that findings were not influenced by results with the irradiated cultures.

**RESULTS**

**Preliminary experiments.** When 1 to 3 μg of Cyt B per ml was added to the growth medium of McCoy cell cultures, definite enlargement of the cells was observed after 1 to 2 days. At that time, many of the cells had 2 to 4 nuclei and were epithelioid rather than spindle shaped. After an additional 1 to 2 days, the cells enlarged further and many of them had 6 to 8 nuclei. These nuclei, though somewhat enlarged, did not develop to the giant size that is commonly seen after irradiation. After prolonged exposure (8 to 10 days), degenerative changes appeared and the cytoplasm disintegrated. When the concentration of Cyt B was increased (10 μg/ml), these degenerative changes appeared earlier, whereas with 0.3 μg of Cyt B per ml only a slight effect on cell morphology was observed.

When pools of T181 were titrated on McCoy cells treated with Cyt B, the cells treated with 1 μg/ml yielded the most inclusions. These inclusions appeared similar in morphology to those formed in irradiated cells, and pretreatment for 72 h before inoculation yielded a comparable number to that found in similarly inoculated irradiated cells. On the basis of these observations, for subsequent work cells were pretreated with 1 μg of Cyt B per ml for 72 h before inoculation. It was found simpler, and equally effective, to add the Cyt B to the cell suspension rather than to individual cell culture tubes of newly formed monolayers. Since certain of the effects of Cyt B are reversible, the same concentration of Cyt B was maintained in the maintenance medium throughout the Chlamydia growth cycle.

**Growth of T181, HAR-1, and LGV II.** Cyt B-treated McCoy cells were at least as effective as irradiated cells for the growth of these three strains of *C. trachomatis*. Table 1 shows that untreated cultures gave significantly lower inclusion counts than cultures treated with Cyt B or irradiation. The counts obtained from Cyt B-treated cultures were higher than from irradiated cultures for all three strains, but the difference was not statistically significant, being in the range of ±1 standard deviation. The morphology of the inclusions in Cyt B-treated and irradiated cells was generally similar, whereas in normal cells the inclusions were smaller, rounder, and "emptier," apparently containing only small numbers of elementary bodies. The LGV strain grown in irradiated cultures for 72 h produced large but still discrete inclusions, whereas in Cyt B-treated cultures many of the inclusions had ruptured by that time. This strain was therefore examined after 30 to 48 h, while the inclusions were still intact, in order to make counting possible.

Positive Cyt B-treated cultures were successfully passed into both irradiated and fresh Cyt B-treated cultures, and the increase in infectivity (10- to 20-fold) was similar to that obtained on passage of positive irradiated cultures.

**Clinical specimens.** Chlamydiae were isolated from 16/51 specimens (31%) in Cyt B-treated cells and from 17/51 specimens (33%) in irradiated cells. There was thus no significant difference between the isolation rates in the two cell systems. Fifteen isolations were made in both types of culture, two in irradiated and not in Cyt B-treated cells and one in Cyt B-treated and not in irradiated cells. When an isolation was made in both systems, the number of inclusions per cover slip found was usually of the same order, and when an isolate was made in only one type of culture the number of inclusions per cover slip was <3.

Five different batches of both irradiated and Cyt B-treated cells were used to test these specimens (about 10 specimens per batch), and the relative sensitivity of the two systems did not fluctuate very much.
TABLE 1. Effect of Cyt B-treatment and irradiation of McCoy cells on the recovery of inclusion-forming units (IU)*/ml from pools of C. trachomatis

<table>
<thead>
<tr>
<th>Chlamydia strain</th>
<th>Treatment of McCoy cell cultures</th>
<th>Cyt B</th>
<th>Irradiation</th>
<th>No treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital TRIC, T181</td>
<td></td>
<td>23</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>No. of cover slips examined</td>
<td></td>
<td>155.7</td>
<td>±11.4</td>
<td></td>
</tr>
<tr>
<td>IU × (4 × 10^9)⁻¹, mean</td>
<td>207.3 ≤ 11.4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SD</td>
<td>±86.4 ≤ 11.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAR-1, serotype A</td>
<td></td>
<td>9</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>No. of cover slips examined</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IU × 4⁻¹, mean</td>
<td>500 ≤ 22.7</td>
<td>463.8</td>
<td>±32.0</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>±99.7 ≤ 32.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>434 serotype LGV II</td>
<td></td>
<td>9</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>No. of cover slips examined</td>
<td></td>
<td>280.8</td>
<td>191.4</td>
<td>27.0</td>
</tr>
<tr>
<td>IU × (4 × 10^9)⁻¹, mean</td>
<td>280.8 ≤ 16.8</td>
<td>191.4</td>
<td>±16.8</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>±58.1 ≤ 16.8</td>
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</tbody>
</table>

* IU was calculated from the number of inclusions per cover slip. SD, standard deviation.

Isolation was confirmed by passage of infectivity from the second culture in all but one instance: the isolation made in Cyt B-treated cells only, where only one isolation was seen. Infectivity was usually increased by about 10-fold on passage in both types of culture.

DISCUSSION

The isolation of chlamydiae is important both for routine diagnosis and for epidemiological studies in trachoma and in nonspecific genital infections, for, since chlamydiae are commonly associated with nongonococcal urethritis, their role in this condition is currently under discussion (2).

Although untreated McCoy cells have recently been used for primary isolation of chlamydiae (6), both the present study, and earlier work by Gordon (4), have demonstrated that irradiated McCoy cells are more sensitive than untreated ones for the growth of these organisms. However, an appropriate source of irradiation is not always readily available to laboratories engaged in isolating chlamydiae.

The Cyt B-treatment described in this study has considerable advantages over irradiation; all procedures can be carried out in the laboratory, sensitized cells can be prepared more quickly, and their number can be more easily adjusted to variable clinical demands. Centrifugation, however, is still an essential part of the isolation technique, whatever type of cell culture is used.

Recently it has been reported that McCoy cells treated with 5-iodo-2-deoxyuridine are as sensitive to C. trachomatis as irradiated cells (13). This report reached us after the present study was completed.

ACKNOWLEDGMENTS

We thank Julius Schacter for supplying two of the Chlamydia strains studied; John D. Trehanr for serotyping isolate T181; A. L. Hilton for collecting the clinical specimens; J. Garrett for irradiating the McCoy cells; and Suzanne K. R. Clarke for helpful advice and criticism. D. S. is grateful to H. R. Cayton and the Public Health Laboratory Service for their hospitality, to Bertha Fris for a stimulating discussion on the effect of cytochalasins on animal cells, and to "The Friends of Assaf Harofe Hospital" who made the stay in Bristol financially possible.

LITERATURE CITED