Isolation of Chlamydia trachomatis in Untreated MMC-E Mouse Epithelial Cells

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Received 3 February 1982/Accepted 29 April 1982

The susceptibility of the epithelial mouse embryo cell line MMC-E to infection with Chlamydia trachomatis was studied by using chlamydiae from both laboratory strain L2 and clinical specimens. MMC-E cells were sensitive to infection with C. trachomatis without any pre- or posttreatments, and their susceptibility for isolation was comparable to that of the conventionally used irradiated McCoy cells.

Chlamydia trachomatis, an obligate intracellular organism, is an important pathogen responsible for several diseases in humans (12, 19, 20). The laboratory diagnosis of chlamydial infections is usually based on the isolation of the organism in irradiated McCoy (IR-McCoy) cells (4, 5), which are closely related to the mouse fibroblastic tumor cell line L929 (1, 4). In addition to the McCoy cell line, many other cell lines are susceptible to Chlamydia (2, 17), but only a few (BHK-21 and HeLa-229) are sufficiently susceptible to be used for isolation of the organism from clinical specimens.

In addition to irradiation, DEAE-dextran, iododeoxyuridine, cytochalasin B, emetine, cytochrome, and hydrocortisone have all been used as alternative methods for treatment of cells to enhance their susceptibility to Chlamydia (3). Primary cell cultures of human or animal thyroid (7, 16), prostate (P. Giles, S. B. Greenberg, S. Doughty, and R. R. Martin, Clin. Res. 26(Suppl. 1):58A, 1978), and amnion (6) have been shown to be as susceptible to Chlamydia as are treated McCoy cells.

MMC-E cells obtained from U. R. Rapp (National Cancer Institute, Frederick, Md.) were grown on plastic tissue culture dishes (Falcon Plastics, Oxnard, Calif.) in BMK medium supplemented with 10% fetal calf serum (Gibco, Renfrewshire, Scotland) and 50 μg of gentamicin (Garamycin; Schering Co., Kenilworth, N.J.) per ml. The stock cells were dispersed with trypsin-EDTA for weekly subculture at a ratio of 1:5. About 3 × 10⁵ cells were seeded in 1.0 ml of growth medium in a flat-bottomed plastic tube (Sterilin Ltd., Middlesex, England) containing a round 13-mm-diameter sterilized cover glass. Tubes were incubated at 35°C in a 5% CO₂ atmosphere and were used for inoculation when cultures became confluent, 1 to 2 days later. McCoy cells were originally purchased from Flow Laboratories, Irvine, Scotland. The preparation of IR-McCoy cell cover slips was done conventionally as previously described (21). C. trachomatis serotype L2 (434Bu) was originally obtained from the Institute of Ophthalmology, London. It was passaged in our laboratory in IR-McCoy cells as described previously (18). The infectivity titer of this stock strain was 5 × 10⁶ inclusion-forming units per ml as determined in IR-McCoy cell cover slip tubes. Serial 10-fold dilutions of the stock strain in 0.2 M sucrose–0.02 M phosphate solution (5) supplemented with 3% fetal calf serum, 50 μg of gentamicin per ml, and 25 U of nystatin (Mycostatin; E. R. Squibb & Sons Inc., Princeton, N.J.) per ml were freshly prepared. The clinical specimens were obtained from patients with cervicitis or nongonococcal urethritis as described previously (13).

All inoculations were done in duplicate in both cell lines. A 0.1-ml amount of each dilution of the stock strain was dispensed into the vials containing confluent layers of untreated MMC-E cells or IR-McCoy cells. Cell monolayers inoculated only with 0.2 M sucrose–0.02 M phosphate solution were used as negative controls. Centrifugation, incubation for 2 days, staining of cover slips with iodine, and counting of iodine-stained inclusions were performed according to standard techniques as described previously (14).

The MMC-E cells remained closely packed and retained a uniform well-spread appearance throughout incubation (Fig. 1A). The largest number of inclusions occurred at the cell density giving a just-confluent cell layer at the time of inoculation (approximately 4 × 10⁵ cells per vial). After iodine staining, the MMC-E cells remained pale yellow and rather translucent, with the nuclei just visible. Thus, the chlamydial inclusions, appearing dark yellow to dark brown, surrounded by the characteristic halo formation, were easily detectable (Fig. 1B). Due to their large size and characteristic appearance,
The cytotoxic effect inclusions of McCoy cells. The highest infectivity MMC-E cover range of were as least as (Fig. L2 artifacts such the inclusions. Dense culture infected with C. trachomatis (iodine staining; phase-contrast micrograph; ×200).

FIG. 1. Photomicrographs of MMC-E cells. (A) Subconfluent uninfected growing culture (×125). (B) Dense culture infected with C. trachomatis (iodine staining; phase-contrast micrograph; ×200).

cell lines, 33 (30%) were positive in IR-McCoy cells, and 34 (31%) were positive in MMC-E cells (Table 1). Of the 34 specimens positive in MMC-E cells, 5 (15%) were negative in IR-McCoy cells. On the other hand, 4 (12%) of the 33 specimens positive in IR-McCoy cells were negative in MMC-E cells. Thus, the sensitivity of the MMC-E cells was comparable to that of IR-McCoy cells (88 versus 85%). With specimens positive in both cell lines, the number of inclusions was generally higher in IR-McCoy cells than in MMC-E cells (Fig. 3).

The establishment of the stable nontumorigenic mouse epithelial cell line MMC-E has made it possible to study in vitro different aspects of diseases that commonly affect epithelial surfaces. The MMC-E cell line was originally established from an embryo of Mus musculus.

The inclusions could be easily differentiated from artifacts such as cell debris.

Titration results of C. trachomatis stock strain L2 (Fig. 2) showed that MMC-E cells were at least as susceptible to C. trachomatis infection as were the IR-McCoy cells over the entire range of inocula tested. In some cases, the MMC-E cover slips gave even greater numbers of inclusions than did the IR-McCoy cover slips. The cytotoxic effect of the inoculum with the highest infectivity was more profound in IR-McCoy cells.

Of 110 clinical specimens inoculated onto both

FIG. 2. Titration curves of C. trachomatis inclusions in IR-McCoy and MMC-E cell lines. Standard C. trachomatis stock was diluted in logarithmic scale, and triplicate cultures of both cell lines were used for each dilution point. The variation in the inclusion numbers of each dilution was less than 10% (means of triplicate samples are plotted). Note the decreased amount of inclusions at the highest dilution in IR-McCoy cultures.

<table>
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<tr>
<th>TABLE 1. Comparison of untreated MMC-E cells and IR-McCoy cells for the isolation of C. trachomatis from clinical specimens</th>
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<tbody>
<tr>
<td>Result in McCoy cells</td>
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<tr>
<td>Positive in MMC-E cells</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
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<td>Total (%)</td>
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*The clinical specimens were thawed if frozen, shaken in a Whirlimixer for 20 s, divided into two equal parts (0.5 ml each), inoculated into the cell cultures, and handled further as described in the text.
MMC-E cells were consistently large and easy to detect. Tubes containing cover slips of untreated cells can be prepared daily. The method presented in this study is readily applicable for routine cultivation of *Chlamydia* in laboratories in which the necessary pretreatments of McCoy cells are too difficult to perform. The cells may also prove useful in studies of the molecular mechanisms involved in the chlamydial infection of nontumorigenic epithelial cells in vitro.

We thank Lea Torvinen and Tuula Nykänen for excellent assistance and Tapani Hovi for valuable discussions.

This study was supported by grants from the Finnish Cultural Foundation, the Finnish Cancer Foundation, Avohoidon Tutkimussäätiö and Labsystems Oy, Helsinki, Finland.

**LITERATURE CITED**


