Isolation of Chlamydia trachomatis by Use of 5-Iodo-2-Deoxyuridine–Treated Cells

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Irradiated McCoy cells have provided a useful technique for the isolation of Chlamydia trachomatis strains, among which are found the etiological agents of trachoma, inclusion conjunctivitis, and lymphogranuloma venereum. Because irradiation is not always readily available, 5-iodo-2-deoxyuridine (IUDR) treatment of cells was investigated as a substitute procedure. IUDR-treated cells were found to be as sensitive to C. trachomatis infection as were irradiated McCoy cells. Stock chlamydial strains gave similar titers of iodine-stained inclusions in either system. When cells treated with IUDR were compared with irradiated cells for the isolation of C. trachomatis from clinical specimens, 5 of 138 specimens yielded isolates in IUDR-treated cells not found in irradiated ones, and one isolate was obtained from irradiated but not from IUDR-treated cells. In those 56 cases where inclusions were seen in both systems, there were significantly more inclusions in IUDR-treated than in irradiated cells. Although this series of cultures is too small to determine whether IUDR-treated cells are superior to irradiated ones for the isolation of C. trachomatis, the data indicate that IUDR treatment is at least equally effective.

The irradiated McCoy cell system has proved to be a useful technique for the isolation of Chlamydia trachomatis strains, among which are found the etiological agents of trachoma, inclusion conjunctivitis, and lymphogranuloma venereum (8). This tissue culture system has been shown to be more sensitive as well as more convenient than the yolk sac technique for the isolation of these organisms from the genital tract, and its use has resulted in several recent studies associating C. trachomatis isolations with nongonococcal urethritis (2, 7, 9) and cervicitis (13). However, some laboratories cannot use this technique because they do not have easy access to an irradiation facility, and unirradiated McCoy cells are relatively insensitive to C. trachomatis infection. We have, therefore, investigated the substitution of a chemical assault upon the cell for that of irradiation. We chose the deoxyribonucleic acid (DNA) analogues 5-iodo-2-deoxyuridine (IUDR) and 5-bromo-2-deoxyuridine (BUDR) for this purpose. Although BUDR treatment of cells appeared to be ineffective at the concentrations tested, IUDR-treated cells were as sensitive to C. trachomatis infection as were irradiated McCoy cells. It appears that IUDR treatment can be easily substituted for irradiation in the isolation of these organisms.

MATERIALS AND METHODS

Cells and media. McCoy mouse heteroploid cells were obtained from G. E. Kenny, University of Washington, Seattle. The cells were grown in Eagle minimum essential medium supplemented with 10% fetal calf sera, 6.6 mM sodium bicarbonate, 5 μg of gentamicin per ml, and 25 U of nystatin per ml (MEM-10). Irradiated cells were overlaid with MEM-10 plus additional glucose (6). Inoculated cells were overlaid with this medium containing 10 μg rather than 5 μg of gentamicin per ml (12) and 20 mM HEPES (β-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) buffer, pH 7.5 (13).

Reagents. The IUDR used in these experiments was obtained from Smith, Kline & French, Philadelphia, Pa., as “Stoxil,” and the BUDR was from Schwarz BioResearch, Inc., Orangeburg, N.Y.

Isolation technique: irradiated McCoy cell system. The irradiated McCoy cell technique used here was that previously described (12, 13), which was based on the methods of Gordon et al. (6). McCoy cells were irradiated with 4,000 to 5,000 roentgens from a cobalt source, incubated for 4 days, harvested with trypsin, and seeded at a concentration of 1.5 × 10⁶ cells per vial into 1-dram (1.177 g) vials containing 12-mm circular cover slips. These vials were incubated 3 days, and the cells were then treated with 30...
µg of diethylaminoethyl-dextran per ml for 30 min prior to inoculation. Treated cells were inoculated with 0.1 ml of specimen material per vial, centrifuged for 1 h at 2,000 rpm, overlaid with 1 ml of medium, and incubated for 3 days before the cover slips were removed for iodine staining (6).

**Isolation technique: analogue-treated cells.**

Cells for IUDR or BUDR treatment were handled in the same manner as cells to be irradiated. Unirradiated cells were treated with analogues for either 8 or 3 days prior to inoculation. Analogue was added to the medium when cells were seeded into vials for the 3 days of treatment. For 8 days of treatment, analogue was placed in the growth medium 5 days before cells were harvested for seeding into vials, as well as in the medium for the seeded vials. Analogue was removed from the cells prior to inoculation, and all inoculated cells were overlaid with the usual medium for irradiated cells.

**Quantitative assays.** Assays of stock strains were based upon counts of iodine-stained inclusions (12). The cells in four vials were inoculated with each dilution of a serially diluted stock strain. The inclusions in 20 randomly chosen fields on each of the four cover slips were counted, and the average number of inclusions per field was calculated. This average was multiplied by the number of fields per cover slip and the appropriate dilution of the inoculum to give a titer in inclusion-forming units.

**Source of specimens.** Cervical and urethral specimens were collected with swabs from patients attending venereal disease clinics. The swabs were transported in the sucrose-phosphate buffer described by Gordon et al. (5) containing 50 µg of streptomycin per ml and 25 U of nystatin per ml. Upon receipt, the specimens were frozen in the laboratory at −70 C and thawed immediately before inoculation.

**Chlamydial stock strains.** Isolates were serially passed in irradiated McCoy cells until inclusion counts indicated titers of 10⁴ to 10⁵ inclusion-forming units. The strains were then harvested in double-strength sucrose-phosphate buffer (5) and stored at −70 C. We are indebted to S. P. Wang, University of Washington, Seattle, for serotyping these strains by his immunofluorescence technique (11). Stock strains B/UW-108/U, C/UW-80/U, D/UW-83/U, E/UW-91/U, F/UW-115/U, G/UW-100/U, FG/UW-89/U, H/UW-99/U, I/UW-114/U, and U-282-1 were isolated from males with urethritis. Strains C/UW-36/Cx, D/UW-169/Cx, and FG/UW-170/Cx were obtained from cervical cultures of females with cervical abnormalities; strains K/UW-71/Cx and D/UW-166/Cx came from cervical cultures of females without evidence of cervical abnormality at the time of culture (13). Strain E/UW-17/Cx was isolated from the cervical culture of a normal pregnant woman.

**RESULTS**

Four cervical isolates (E/UW-17/Cx, D/UW-169/Cx, FG/UW-170/Cx, D/UW-166/Cx) and two urethral isolates (U-282-1, FG/UW-89/U) were assayed in three different cell systems—irradiated McCoy; unirradiated, untreated McCoy; and unirradiated McCoy treated with 50 µg of BUDR per ml for 3 days prior to inoculation. In all cases the titer in irradiated McCoy cells was significantly higher than the titer in either of the other systems (P < 0.001), and in five of the six cases, the titer in unirradiated, untreated cells was significantly higher than that in BUDR-treated ones (P < 0.001).

Because it was considered that 50 µg of BUDR per ml for 3 days might not be sufficient for cell treatment, strain FG/UW-89/U was assayed in the same fashion in cells treated for 8 days with 50 or 100 µg of BUDR per ml and in cells treated with an intermediate concentration of 80 µg of IUDR per ml. Only the titer in IUDR-treated cells was equal to or higher than the titer in irradiated cells (P < 0.001).

Because IUDR at this concentration appeared to be effective in sensitizing cells to infection with this strain, cells treated with 80 µg of IUDR per ml for only 3 days were used to assay 11 strains of different serotypes and the titers were compared to assays in irradiated cells (Table 1). Titters in the two systems were similar for all strains, and there were no obvious associations between serotype or source and method of assay. Inclusion counts for IUDR-treated cells were significantly higher than those for irradiated cells in five instances and significantly lower in three (P < 0.05).

Three strains were serially propagated from IUDR-treated cells to IUDR-treated cells and to irradiated cells, and from irradiated cells to both IUDR-treated and to irradiated cells. Material from IUDR-treated cells was as infective as material from irradiated cells, and either cell system was suitable for serial propagation of strains.

**Table 1. Assay of 11 chlamydial strains in irradiated and IUDR-treated McCoy cells**

| Strain | Irradiated cells | IUDR-treated cellsa |
|--------|-----------------|---------------------|
| B/UW-108/U | 1.40 × 10⁴ | 1.83 × 10⁶ |
| C/UW-80/U | 5.17 × 10⁴ | 3.93 × 10⁶ |
| C/UW-36/Cx | 3.01 × 10⁵ | 3.40 × 10⁶ |
| D/UW-83/U | 6.86 × 10⁴ | 8.85 × 10⁶ |
| E/UW-91/U | 5.80 × 10⁴ | 5.39 × 10⁶ |
| ED/UW-111/U | 8.13 × 10⁴ | 9.62 × 10⁶ |
| F/UW-115/U | 1.95 × 10⁴ | 1.55 × 10⁶ |
| G/UW-100/U | 5.03 × 10⁴ | 8.07 × 10⁶ |
| H/UW-99/U | 1.23 × 10⁴ | 1.50 × 10⁶ |
| I/UW-114/U | 6.10 × 10⁴ | 8.58 × 10⁶ |
| K/UW-71/Cx | 5.19 × 10⁴ | 4.32 × 10⁶ |

a 80 µg of IUDR per ml for 3 days prior to inoculation.

b Inclusion-forming units.
Further experiments were done to determine an optimal concentration and time for IUDR treatment of cells. Cells treated 3 or 8 days prior to inoculation were treated with a range of concentrations (25 to 200 μg of IUDR per ml) (Table 2) and examined 72 h postinoculation. These data suggested that higher concentrations of IUDR had an inhibitory effect, because inclusion counts decreased with increasing amounts of IUDR, regardless of the length of treatment. Three days of treatment appeared equal or superior to 8 days, and both gave titers higher than those in irradiated cells even at concentrations of 100 μg of IUDR per ml.

The same two chlamydial strains were then assayed in cells treated with lesser concentrations of IUDR for 3 days (Table 3). Inclusion counts were done at 24, 48, and 72 h postinfection. No iodine-stained inclusions were seen after 24 h, and inclusions were generally smaller, somewhat less frequent, and more difficult to count at 48 than at 72 h postinfection. Inclusions in cells treated with 5 μg per ml were also somewhat more difficult to count than those in cells treated with higher concentrations of IUDR, and 10 to 25 μg per ml appeared to be optimal for treatment in terms of inclusion counts and appearance of inclusions.

Cells treated with 25 μg of IUDR per ml for 3 days were compared with cells treated for only 2 days prior to infection for assay of 10 chlamydial strains. Two of these strains produced inclusions in the 3-day- but not in the 2-day-treated cells. Six strains produced more inclusions in 3-day-treated cells than in 2-day-treated cells, and two strains gave approximately the same number of inclusions in both systems. Because two days of treatment did not appear to be sufficient for optimal sensitization of cells and the results at 3 and 8 days of treatment were not greatly different, no intermediate times were tested and 3 days was chosen as the standard time for treatment.

Cells treated with 25 μg of IUDR per ml for 3 days were then compared with irradiated cells for the isolation of C. trachomatis strains. A total of 138 specimens, which included 89 cervical, 46 urethral, and 3 cultures from other sites, were inoculated in parallel into IUDR-treated and into irradiated cells. One cervical specimen was found to be unsatisfactory in both systems due to the presence of other contaminating organisms. Five specimens yielded chlamydial isolations in the IUDR-treated cells not found in the irradiated cells (Table 4), and one isolate obtained from irradiated cells was not found in IUDR-treated ones. In those 56 cases where inclusions were seen in both systems, there were significantly more inclusions seen in the IUDR-treated cells than in the irradiated ones (P < 0.001).

Although this series of cultures is too small to determine whether IUDR-treated cells are superior to irradiated ones for the isolation of C. trachomatis, IUDR treatment appears to be at least as effective as irradiation.

**DISCUSSION**

Gordon et al. (6) hypothesized that increased sensitivity to C. trachomatis infection after irradiation of McCoy cells was due to an increase in the presence of multinuclear giant cells, which constituted the sensitive cell of unirradiated McCoy cell monolayers. Unirradiated McCoy cells have a limited sensitivity to infection, presumably due to the few giant cells present normally. These workers determined the relative size of irradiated and unirradiated McCoy cells and found that irradiation increased the average cell size. No such studies have been done on IUDR-treated McCoy cells.

It has been shown that the thymidine analogues, BUDR and IUDR, can induce the production of Epstein-Barr virus in nonproducer cells (4), and IUDR renders nonpermissive epithelioid cells permissive to cytomegalovirus infection (10). IUDR and BUDR not only interact with the enzymes that synthesize DNA precursors, but these analogues also are converted to the triphosphates that can be incorporated into DNA in place of thymidine. The resultant DNA may continue to replicate and cause the synthesis of nonfunctional proteins (1). The work of Friis with Chlamydia psittaci (3) suggests that

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**Table 2. Assay of two chlamydial strains in cells treated with varying concentrations of IUDR**

| Strains | IUDR (μg/ml) | Time of treatment |
|---------|-------------|------------------|
|         |             | 3 days | 8 days |
| C/UW-80/U | 25          | 1.85×10⁴ | 1.55×10⁴ |
|          | 50          | 1.85×10⁴ | 1.27×10⁴ |
|          | 80          | 7.09×10⁴ | 9.10×10³ |
|          | 100         | 1.24×10⁵ | 9.03×10³ |
|          | 200         | 5.33×10⁵ | 4.15×10³ |
| E/UW-91/U | 25          | 4.16×10⁴ | 3.90×10⁴ |
|          | 50          | 3.37×10⁵ | 2.82×10⁴ |
|          | 80          | 3.64×10⁵ | 2.34×10⁴ |
|          | 100         | 3.45×10⁵ | 2.18×10⁴ |
|          | 200         | 5.33×10⁵ | 4.15×10³ |

* C/UW-80/U and E/UW-91/U without IUDR treatment had 1.12×10⁴ and 2.39×10⁴ inclusion-forming units, respectively.

* Inclusion-forming units, 72 h postinoculation.
some intrinsic property of this agent prevents the usual sequence of host lysosome response that results in the degradation of heat-inactivated C. psittaci. If this same degradative mechanism prevents replication of C. trachomatis in untreated McCoy cells, irradiation or IUDR treatment may in some way interfere with the function of this system. Irradiation is known to interfere with DNA replication and transcription. Because IUDR is incorporated into DNA, its effect on DNA transcription may lead to an altered RNA, whose translation results in nonfunctional enzymes in the cell’s degradative mechanism. The treated cell now permits the continued presence and replication of an agent that it normally would degrade.

It appears that incorporation of analogue into DNA is not the essential step for sensitizing a cell to C. trachomatis infection, because BUDR, which can be incorporated, was not an effective sensitizing agent at the concentrations tested. It is possible that smaller concentrations of this analogue may be more effective in sensitizing cells. IUDR has a more extensive impact on cellular functions than does BUDR, which may account for the difference in their behavior in these studies.

Although the mechanism of the sensitizing effect is not known, it appears that IUDR can be used to replace irradiation of McCoy cells for the isolation of C. trachomatis. The number of specimens examined in parallel here was not large, and the rate of chlamydial isolations in IUDR-treated cells was not significantly greater than that in irradiated cells. However, the results indicate that IUDR treatment, which is considerably simpler than irradiation, yields at least equally good results. Therefore, any laboratory with facilities for tissue culture can readily adapt its techniques to isolation of these agents.

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