Aberrant Regulation of Ornithine Decarboxylase by Serum, Putrescine, and Spermidine in Cytomegalovirus-transformed Human Cells

Harriet C. Isom and Jay T. Backstrom

ABSTRACT

Regulation of ornithine decarboxylase (ODC) activity by serum and polyamines in cytomegalovirus (CMV)-infected and CMV-infected cells, and in early- and late-passage CMV-transformed human cells was studied. Exposure of early-passage growth-arrested CMV-transformed human cells to fresh medium containing 10% serum resulted in dramatic but short-lived elevations in ODC activity; maximal activity was approximately 40-fold greater than that in serum-stimulated normal human cells and occurred 5.5 hr after the addition of fresh serum. The response of ODC activity in late-passage CMV-transformed cells to fresh 10% serum-containing medium was only about 10-fold greater than that in normal human cells.

In general, polyamines in the growth medium were less effective in reducing ODC activity in CMV-transformed cells than in normal cells, but the specific effect varied with the polyamine and with the transformed cell passage level. Growth of cells in 10⁻⁶ M spermidine reduced ODC activity 94% in normal human cells, 54% in late-passage CMV-transformed cells, and 15% in CMV-infected cells, but spermidine was not inhibitory to early-passage CMV-transformed cells. Early-passage CMV-transformed cells were as sensitive to growth in putrescine as were normal cells, while late-passage CMV-transformed cells were completely resistant to 10⁻⁶ M putrescine. The stimulation of ODC activity seen after CMV infection of normal human cells was not seen after CMV infection of CMV-transformed cells.

INTRODUCTION

CMV has many properties that implicate it as an oncogenic virus such as the ability to stimulate host cell DNA (6, 7, 32, 33), RNA (35), and protein (31) synthesis and to transform cells in culture (1, 11). The original work of Albrecht and Rapp (1) showed that UV-irradiated human CMV retains the ability to transform hamster embryo fibroblasts and that these transformed cells are oncogenic in newborn hamsters. More recently, Geder et al. (11) demonstrated the first long-term human herpesvirus transformation of human cells in primary culture by transforming human embryo lung cells with a noninactivated genital CMV (CMV-Mj) isolated from human prostatic cells (29).

The transformed cells produced tumors when injected into athymic nude mice, and cells cultured from one of these tumors (CMV-Mj-HEL-2,T-1) have been studied at increasing passage level for oncogenicity and expression of CMV antigens (10, 11, 28). Support for the theory that human CMV may induce neoplasias comes from findings that infection not only stimulates host cell DNA synthesis but also stimulates enzymes, possibly related to DNA synthesis, such as thymidine kinase (5, 40), DNA polymerase (14, 15), and ODC (18). Stimulation of ODC activity is also seen after infection of arrested hamster cells with UV-irradiated CMV (17). Dramatic elevations in ODC activity accompany transformation by several animal viruses (3, 4, 9). Elevation of ODC activity also accompanies spontaneous (38) and chemically induced (25) neoplasia and occurs shortly after infection, precedes morphological transformation, and, as such, may be an early metabolic alteration related to cancer and loss of growth control.

We have shown in a previous report (18) that infection of human cells with human CMV causes a marked increase in ODC activity, and we were interested in determining (a) whether this elevation is fixed by transformation and (b) whether ODC in CMV-transformed cells is subject to the same regulation by serum and polyamines that is observed in normal human cells.

MATERIALS AND METHODS

Cells. Whole human embryo (Flow 5000) cells, purchased commercially from Flow Laboratories, Inc. (Rockville, Md.), were subcultured at a ratio of 1:2 using trypsin and were grown in 75-sq cm plastic flasks (Falcon Plastics, Oxnard, Calif.) in minimum essential medium supplemented with 10% FCS, 0.075% NaHCO₃, 100 units of penicillin per ml, 100 μg of streptomycin per ml, 1 μg of Fungizone per ml, and 10 units of Mycostatin per ml. CMV-Mj-HEL-2,T-1 cells were kindly provided by Dr. L. Geder (The Milton S. Hershey Medical Center, Hershey, Pa.). CMV-Mj-HEL-2,T-1 cells were derived from a tumor produced after inoculating a nude mouse with human embryonic lungs cells transformed by CMV-Mj and are referred to in the paper as CMV-transformed cells. CMV-transformed cells were subcultured at a ratio of 1:4 using trypsin (0.125%) and Versene (0.02%) and were grown in 75-sq cm plastic flasks in Ham’s F10 medium supplemented with 20% FCS, 0.075% NaHCO₃, and antibiotics. Early-passage CMV-Mj-HEL-2,T-1 cells refer to passages 21 to 35 while late-passage cells represent passages 219 to 238. Normal human embryo cells and both early- and late-passage CMV-transformed cells were tested (8) and were found...
to be free of Mycoplasma contamination. CMV-transformed cells derived from tumors were used for these studies instead of the original transformants in order to eliminate Mycoplasma contamination.

Preparation of Virus and Mock Infesting Fluid. Human CMV strain AD169 was used, and stocks were prepared in Flow 5000 cells. Cells were infected at a virus:cell ratio of 0.2 and were fed maintenance medium (2% FCS) at 2-day intervals. Virus was harvested based on cytopathology at 7 to 10 days postinfection by freezing and thawing, sonication, and clarifying by centrifugation. Mock infesting fluid was prepared from Flow 5000 cells by the procedure used for preparation of CMV. CMV titer was measured in Flow 5000 cells by the plaque assay described by Wentworth and French (36).

Preparation of Arrested Cells. In order to reduce the background level of ODC, cells were arrested by a combination of growth to confluence and depletion of serum prior to the initiation of experiments. Normal human cells were subcultured 1:2 in 150-sq cm plastic Petri dishes (Corning Glass, Corning, N. Y.) and grown to confluence (2 to 3 days) in 10% FCS medium. Cultures were then washed in medium lacking serum, fed 0.2% FCS medium, and maintained in this medium for 48 hr prior to use. Cells prepared in this manner are referred to in the text as arrested cells. In experiments in which putrescine, spermidine, or 1,3-diaminopropane were added to the growth medium, cells were arrested in 0.2% horse serum medium instead of 0.2% FCS medium. Early-passage CMV-Mj-HEL-2,T-1 cells were prepared in the same manner except that subculture was at a ratio of 1:4. Late-passage CMV-Mj-HEL-2,T-1 cells were also passaged 1:4 but were fed 0.5% FCS medium since they did not survive when fed only 0.2% FCS medium.

To initiate an experiment, 0.2% or 0.5% serum medium was removed from arrested cells and replaced with fresh 10% serum-containing medium. Addition of this medium is referred to as high-serum shift. Arrested cell cultures to which no additions were made were harvested with each experiment and assayed for enzyme activity in order to control for the arrest procedure.

Preparation of Extract for ODC Assay. Cells were seeded in large tissue culture dishes (150 sq cm). Two dishes of cells were used to determine each specific activity. At the time of harvest, medium was removed, the cells were washed in phosphate-buffered saline (0.12 M NaCl:12 mM Na2HPO4:1.5 mM KH2PO4), and the dishes were thoroughly drained to remove any residual phosphate-buffered saline. A 1-ml volume of disruption buffer containing 25 μmol Tris-HCl (pH 7.1), 0.05 μmol EDTA, 0.025 μmol pyridoxal 5’-phosphate, and 2.5 μmol dithiothreitol (9) was added, and the cultures were frozen and thawed 3 times. The thawed lysate was scraped from each plate with a rubber policeman, pooled, and centrifuged at 12,000 X g for 20 min. Supernatant fractions of approximately 2 ml each were stored at -70° until assayed. Fractions retained stable ODC activity for at least 10 days but were routinely assayed 24 to 48 hr after preparation.

ODC Activity. Enzyme activity was determined by measuring the release of 14CO2 from DL-[1-14C]ornithine (New England Nuclear, Boston, Mass.) as described (20) with the following modifications. Incubations were carried out in serum bottles (30 ml; Wheaton, Millville, N. J.) equipped with rubber stoppers supporting a polypropylene center well (Kontes Glass Co., Vineland, N. J.). Each assay contained 0.5 μCi DL-[1-14C]ornithine (4 mCi/mmol). 0.250 to 0.500 μl supernatant, and disruption buffer in a final volume of 0.55 ml. Released 14CO2 was trapped in 0.2 ml of Hyamine hydroxide contained in the center well. After 60 min or the stated time of incubation at 37° in a shaking water bath, the reaction was stopped by injecting 0.5 ml of 5 H2SO4 into the reaction mixture through the rubber cap. Agitation was continued for an additional 60 min to ensure complete absorption of 14CO2. The center well was removed and placed in a 10-ml liquid scintillator (New England Nuclear Formula 949) for counting. Values obtained were corrected against values for boiled supernatant. One unit of enzyme activity is defined as 1 nmol of CO2 released per hr. Protein concentration was measured using the BioRad protein determination kit (BioRad Laboratories, Rockville Centre, N. Y.) with crystalline bovine γ-globulin as a standard. Specific activity for each culture was determined from duplicate assays at 2 different protein concentrations.

RESULTS

High-Serum Stimulation of ODC Activity in Normal and CMV-transformed Human Cells. Quiescent cells in culture have low levels of ODC activity (34). Allowing some cell types such as WI-38 cells to grow to confluence practically eliminates incorporation of [3H]thymidine into DNA (37); for other cell types such as BHK21/C13 cells, density-dependent inhibition leads to only a gradual decrease in DNA synthesis, and serum deprivation is necessary to produce the desired reduction in the number of cells synthesizing DNA (16, 23). In these studies, we arrested cells using a combination of confluence and serum deprivation. The ODC specific activities of extract prepared from cultures of normal human or early-passage CMV-transformed cells, grown to confluence and subsequently fed 0.2% serum-containing medium for 48 hr, were 0.015 to 0.025 and 0.015 to 0.027 nmol 14CO2 per mg per hr, respectively. The range of activity was obtained from 3 independent experiments. When medium supplemented with 10% FCS was added to arrested cultures, induction of ODC activity occurred in both normal and transformed human cells (Chart 1), but the magnitude and time course of stimulation were considerably different. Maximum stimulation in early-passage CMV-transformed cells exceeded that in normal human cells by 40-fold. The elevated activity in transformed cells was short-lived and considerably reduced by 8 hr after serum addition. By 16 hr, ODC activity in early-passage-transformed cells was only 25% of that in normal human cells. Stimulation in normal human cells persisted and remained at 79% of the maximum as late as 16 hr after serum addition (Chart 1A).

We were also interested in establishing whether ODC activity and the nature of its response to changes in serum concentration were retained with passage in CMV-transformed cells. Unlike normal or early-passage CMV-transformed human cells, late-passage CMV-transformed cells could not be reversibly arrested by use of 0.2% serum-
Chart 1. Stimulation of ODC activity by high-serum shift in normal human (A) and CMV-transformed (B) cells. At Time 0, culture medium was removed from arrested cells and replaced with high-serum (10% FCS) medium. Cultures were harvested at various times after medium change and were assayed for ODC activity. ■, normal human cells; □, early-passage CMV-transformed cells; ●, late-passage CMV-transformed cells containing medium. Within 24 hr of feeding cultures medium containing only 0.2% serum, late-passage cells became granular and were no longer adherent. Further examination of ODC activity in late-passage CMV-transformed cells revealed that a reversible quiescent state could be established if confluent cells were fed 0.5% instead of 0.2% FCS medium. As shown in Chart 2, confluent late-passage cells (Day 0) had an ODC specific activity of approximately 0.09 nmol ^14CO_2 per mg per hr. On Day 1 after subculture, activity peaked at 1.4 nmol ^14CO_2 per mg per hr. The activity then declined to a low level since confluence was reached on Day 3. Feeding with 0.5% FCS medium for 24 hr reduced the activity to the base levels seen in serum-deprived confluent normal or early-passage CMV-transformed cells. Further reduction in activity was not found if serum deprivation was extended to 48 hr (data not shown). However, after either 24 or 48 hr in 0.5% serum medium, the cells responded equally well to a 6-hr high-serum stimulation. As shown, it was also possible to stimulate Day 2 late-passage cells by replacement of the culture fluid with fresh serum-containing medium for 6 hr. Maximum serum stimulation of ODC activity in arrested late-passage CMV-transformed human cells was approximately 10-fold greater than serum-stimulated ODC activity in normal human cells (Chart 1B).

Regulation by Diamines and Polyamines in Early-Passage CMV-transformed Cells. One current theory is that the amount of ODC protein in animal tissues and in cells in culture is largely controlled by putrescine and spermidine through a repression mechanism (2, 19, 21). It has also been shown that "gratuitous" repression of ODC can be produced in vivo by giving partially hepatectomized rats injections of 1,3-diaminopropane, a structural analog of putrescine (27). Repression of ODC in cells in culture by putrescine and spermidine requires the addition of only μM concentrations of these compounds. Because ODC levels are low in nondividing tissue or cells, repression by polyamines or diamines is most easily studied by experimentally stimulating ODC activity and then assessing the ability of these compounds to prevent stimulation. Growth of early-passage CMV-transformed human cells in a 10^{-4} M concentration of putrescine, spermidine, and 1,3-diaminopropane inhibited ODC activity (Chart 3) to 10% or less of the uninhibited control; however, no reduction was observed after growth in 10^{-6} M spermidine.

At high concentrations, polyamines directly inhibit mammalian cell ODC activity, but concentrations of 10^{-4} M or greater are required to observe such inhibition. To eliminate the possibility that the effects observed were caused by direct inhibition of enzyme activity, extract prepared from early-passage CMV-transformed cells was incubated in its reaction mixture with various concentrations of polyamines (10^{-4} to 10^{-6} M), and the ODC activity was measured. Addition of 10^{-4} M spermidine or 1,3-diaminopropane directly to the reaction mixture had no effect on ODC activity; addition of 10^{-4} M putrescine competitively inhibited activity but only by 20%.

Regulation by Spermidine in CMV-infected Human Cells and in Early- and Late-Passage CMV-transformed Cells. We have previously reported that infection with human CMV stimulates ODC activity, that this stimulation is not inhibited by growth in putrescine even at concentrations of 10^{-4} M, and in fact, that ODC activity is 30% greater in the presence of both virus and putrescine than with virus alone (18). Since ODC activity was not reduced by growth of early-passage CMV-transformed cells in 10^{-6} M spermidine, the effect of growth in spermidine on stimulation of ODC in normal cells infected with CMV was examined. CMV-induced stimulation of ODC was partially inhibited by spermidine (Table 1) but was significantly less sensitive than high-serum-induced stimulation of the enzyme to growth in spermidine. At 10^{-6} M spermidine, high-serum-induced stimulation was inhibited by 94%, while the elevation produced by CMV infection was reduced only 15%.

Stimulation of ODC activity in late-passage CMV-transformed cells was inhibited by 54% when 10^{-6} M spermidine...
Effect of spermidine on high-serum- and CMV-transformed cells by growth in di- and polyamines. Arrested low-passage CMV stimulation of ODC.

Horse serum medium supplemented with varying concentrations of putrescine (C), spermidine (M), and 1,3-diaminopropane (W). ODC activity in unsupplemented cultures was used as the control.

Table 1: Inhibition of ODC activity in low-passage CMV-transformed human cells by growth in di- and polyamines. Arrested low-passage CMV-transformed human cells were exposed to a 5.5-hr high-serum shift into 10% horse serum medium supplemented with varying concentrations of putrescine (C), spermidine (M), and 1,3-diaminopropane (W). ODC activity in unsupplemented cultures was used as the control.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Spermidine addeda (M)</th>
<th>ODC activity (nmol ¹⁴CO₂/mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrested normal human cells</td>
<td></td>
<td>0.025 ± 0.002b</td>
</tr>
<tr>
<td>CMV-infected normal human cells</td>
<td>10⁻¹</td>
<td>0.095 ± 0.004 (100)c</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>0.080 ± 0.008 (84.5)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>0.061 ± 0.003 (63.8)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁶</td>
<td>0.052 ± 0.002 (54.5)</td>
</tr>
<tr>
<td>High-serum shift on normal human cells</td>
<td>10⁻¹</td>
<td>0.400 ± 0.001 (100)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>0.024 ± 0.001 (6.0)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>0.027 ± 0.001 (6.8)</td>
</tr>
<tr>
<td>High-serum shift on early-passage CMV-transformed cells</td>
<td>10⁻¹</td>
<td>5.53 ± 0.25 (100)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>6.29 ± 0.09 (114)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>0.755 ± 0.058 (14)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁶</td>
<td>0.580 ± 0.015 (11)</td>
</tr>
<tr>
<td>High-serum shift on late-passage CMV-transformed cells</td>
<td>10⁻¹</td>
<td>2.61 ± 0.46 (100)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>1.19 ± 0.04 (46)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>0.248 ± 0.006 (10)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁶</td>
<td>0.248 ± 0.016 (10)</td>
</tr>
</tbody>
</table>

a Spermidine was added after adsorption of the virus in infected cells and with medium change in high-serum-shifted cells.

b Mean ± S.D.

c CMV infection was for 24 hr; stimulation was 6.5-fold that in the mock-infected control.

d Numbers in parentheses, percentage.

e High-serum shift was for 5.5 hr in the presence of 10% horse serum.

was added to the growth medium (Table 1), indicating that ODC in late-passage CMV-transformed cells is not as resistant to inhibition by spermidine as it is in early-passage CMV-transformed cells but is significantly more resistant than ODC in normal human cells.

Regulation in Early- and Late-Passage CMV-transformed Cells by Putrescine. The effect of growth in putrescine on high-serum stimulation of ODC activity in early-passage CMV-transformed cells was similar to that in normal human cells with significant inhibition in 10⁻⁴ M putrescine (Table 2). In contrast, in late-passage CMV-transformed cells, growth in 10⁻⁴ M putrescine had no effect on stimulation of ODC.

Effect of Superinfection with Human CMV of CMV-transformed Cells. Since infection of normal human cells with CMV leads to a stimulation of cell ODC activity, it was of interest to determine whether ODC in CMV-transformed cells could be similarly induced. Early-passage CMV-transformed cells were arrested in low serum and then mock infected or infected for 16, 24, and 32 hr at a multiplicity of 10 PFU/cell. Although CMV-transformed cells were obtained by transformation with CMV-Mj, CMV AD169 was used for superinfection because the Mj strain could be grown to maximum titers of only 10⁶ PFU/ml, whereas strain AD169 could be grown to titers of 2 × 10⁷ PFU/ml. It was not possible to infect a sufficient number of cells to measure ODC activity using the Mj strain. Enzyme activity in cultures exposed to human CMV AD169 did not exceed the activity in cultures exposed to mock infecting fluid (Table 3). We also noted that ODC activity in mock-infected CMV-transformed cells was 10-fold greater than that in mock-infected normal human cells.

ODC stimulation, which follows CMV infection of normal human cells, was previously shown to require a virus function (18). Since CMV-transformed human cells infected with CMV AD169 at a multiplicity of 5 PFU/cell fail to synthesize virus DNA or grow virus (28), the lack of increase in ODC activity was most probably due to resistance to superinfection.

**DISCUSSION**

Recent studies with the mouse skin carcinogenesis system have shown that, at the metabolic level, one of the earliest and greatest responses to promotion by phorbol diesters is the induction of ODC (22, 24, 26, 39). It is possible that: (a) induction of ODC is simply a marker of the promotion of carcinogenesis; or (b) alterations in regulation of ODC and subsequent changes in polyamine levels are necessary metabolic intermediates between the initiation of tumor formation and the loss of growth control in malignant cells. Indeed, it has been shown that, although a high positive correlation exists between ODC activity and growth rate in rat brain tumor cells in culture (12), hepatomas (38), mouse L1210 lymphoid leukemia (30), and Ehrlich ascites carcinoma (13), not all human and animal tumors have high ODC levels (34).

Initiation of transformation by oncogenic virus genetic information is accompanied by changes in cell metabolism and most probably by changes in regulation of metabolic enzymes. Some of these alterations may be transiently expressed only during initiation, some may be stably retained during maintenance of transformation, while others may fluctuate during maintenance. We have previously shown that infection by human CMV stimulates ODC activity and alters the regulation of human cell ODC such that the total enzyme activity is no longer altered by putrescine in the culture medium (18). In this report, studies on regulation of ODC by polyamines in CMV-infected human cells have been continued and extended to CMV-transformed human cells. When spermidine instead of putrescine was added to the growth medium, stimulation of human cell ODC by human CMV was partially inhibited, but higher spermidine concentrations were required for this inhibition.
Table 2
Regulation of ODC activity in normal and CMV-transformed human cells by putrescine

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Human cells</th>
<th>CMV-Mj-HEL-2,T-1, passage 30</th>
<th>CMV-Mj-HEL-2,T-1, passage 230</th>
</tr>
</thead>
<tbody>
<tr>
<td>High serum^a</td>
<td>0.400 ± 0.009^b (100)^c</td>
<td>13.1 ± 0.10 (100)</td>
<td>1.46 ± 0.01 (100)</td>
</tr>
<tr>
<td>+10^-6 M putrescine</td>
<td>0.211 ± 0.017 (53)</td>
<td>4.71 ± 0.17 (36)</td>
<td>1.73 ± 0.05 (118)</td>
</tr>
<tr>
<td>+10^-4 M putrescine</td>
<td>0.137 ± 0.001 (34)</td>
<td>0.942 ± 0.009 (4)</td>
<td>0.006 ± 0.018 (6)</td>
</tr>
</tbody>
</table>

^a Arrested cells were fed fresh medium containing 10% horse serum and harvested 5.5 hr after medium change.
^b Mean ± S.D.
^c Numbers in parentheses, percentage.
^d ND, not determined.

Table 3
ODC activity in early-passage CMV-Mj-HEL-2,T-1 cells after infection with CMV or mock infecting fluid

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Time postinfection (hr)</th>
<th>ODC activity (nmol [14C]CO_2/mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV^a</td>
<td>16</td>
<td>0.113 ± 0.010^b</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.327 ± 0.095 ± S.D.</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0.159 ± 0.039 ± S.D.</td>
</tr>
<tr>
<td>Mock infecting fluid</td>
<td>16</td>
<td>0.152 ± 0.010 ± S.D.</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.327 ± 0.095 ± S.D.</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0.159 ± 0.039 ± S.D.</td>
</tr>
</tbody>
</table>

^a Cells were infected at a multiplicity of 10 PFU/cell.
^b Mean ± S.D.

than when the enzyme was stimulated by high serum. In CMV-transformed cells, the addition of fresh high-serum medium stimulated ODC activity to higher levels than in normal cells, and growth in either putrescine or spermidine had different inhibitory effects on ODC activity than in normal cells. Differences were most apparent when cells were grown in polyamines at a concentration of 10^-6 M. ODC activities in both early- and late-passage CMV-transformed cells were less sensitive to inhibition by growth in 10^-6 M spermidine than activity in normal cells, with early-passage cells being completely resistant to 10^-6 M spermidine. Putrescine (10^-4 M) inhibited ODC activities in early-passage CMV-transformed cells and normal cells equally but did not inhibit late-passage cells. We can conclude from the present study that transformation of human cells by CMV is accompanied by significant changes in stimulation of ODC activity by exposure to fresh high-serum medium and inhibition of ODC by growth in the polyamines putrescine and spermidine. These alterations in regulation reflect those seen after infection and are unstable with cell passage.

The CMV-transformed human cells used in this study have been subcultured in vitro for over 300 passages. Biological studies by Rapp and Geder (28) have shown that, with passage, CMV antigens in CMV-transformed cells disappear while tumorigenicity reappears. Between passages 20 and 146, CMV-transformed cells produce no tumors in nude mice, whereas cells from passage levels greater than 146 produced tumors in 66% of the mice given injections. We have found that regulation of ODC activity produced by high serum and growth in polyamines also varies with passage. In late-passage CMV-transformed cells compared to early-passage cells: (a) maximum ODC activity following high-serum shift was diminished; (b) inhibition by putrescine was completely lost; and (c) inhibition by spermidine reappeared. Without further investigation, it is not possible to conclude whether the differences in regulation of ODC with cell passage that we have seen are related to the differences in expression of CMV antigens and tumorigenicity seen by Rapp and Geder (28).

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REFERENCES


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