Influence of SV40 Genome on the Replication of an Adenovirus-SV40 “Hybrid” Population

L. A. FELDMAN, J. L. MELNICK, AND F. RAPP

Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

Received for publication 26 April 1965

ABSTRACT

FELDMAN, L. A. (Baylor University College of Medicine, Houston, Tex.), J. L. MELNICK, AND F. RAPP. Influence of SV40 genome on the replication of an adenovirus-SV40 “hybrid” population. J. Bacteriol. 90:778-782. 1965—Replication of a type 7 adenovirus-SV40 hybrid population in primary African green monkey kidney cells was accompanied by the formation of SV40 tumor antigen, adenovirus antigens, and cytopathic changes characteristic of adenovirus infection. Prior infection of the cultures with SV40 stimulated replication of nonintegrated adenovirus 7 but did not enhance the replication of the hybrid virus. These results suggest that the population of the adenovirus-SV40 hybrid studied contains many particles carrying SV40 information. Replication of SV40 virus was not enhanced by co-infection with nonintegrated adenovirus 7 or with the adenovirus-SV40 hybrid. Cytosine arabinoside strongly inhibited replication of the adenovirus-SV40 hybrid population in African green monkey kidney cells. Enhanced replication of nonintegrated adenovirus 7 by SV40 was blocked by cytosine arabinoside; this block could be reversed by 2-deoxycytidine or deoxycytidine triphosphate.

Certain preparations of adenovirus type 7 once grown in monkey-kidney cultures contaminated with papovavirus SV40 seem to have incorporated a portion of the SV40 genome (Huebner et al., 1964; Rowe and Baum, 1964; Rapp et al., 1964a). This genome is expressed by the synthesis of SV40 tumor or T antigen, similar to or identical with the T antigen induced by SV40 during the growth cycle of the virus (Sabin and Koch, 1964; Rapp et al., 1964b; Hoggan et al., 1965) and in cells transformed by SV40 (Black et al., 1963; Rapp et al., 1964a; Pope and Rowe, 1964). SV40 viral antigens are not induced by the adenovirus-SV40 hybrid population, however.

Observations that the deoxyribonucleic acid antagonist, cytosine arabinoside (CA), inhibits the synthesis of SV40 virus (Rapp, Melnick, and Kitahara, 1965b; Rapp et al., 1965a; Melnick and Rapp, in press) but is ineffective in inhibiting the replication of adenoviruses (Butala, 1964) suggested that studies on the effect of this compound on the replication of the adenovirus-SV40 hybrid population might yield additional information concerning the nature and extent of integration. Inoculation of the cultures with SV40 prior to infection with adenovirus was also investigated, since previous reports had revealed enhancement of the replication of adenovirus types 5 and 12 (O’Conor et al., 1963; Rabson et al., 1964) following such a procedure. It was hoped that the results of this study would yield data bearing on the genetic makeup of the adenovirus-SV40 hybrid population.

MATERIALS AND METHODS

Viruses. Nonintegrated adenovirus type 7 was isolated from a fatal case of pneumonia and disseminated disease (Benyesh-Melnick and Rosenberg, 1964). The virus has been serially passed three times in human embryonic kidney cultures (HEK) and has not been passed in cells of nonhuman origin. The strain of SV40 used in these experiments was the reference Baylor strain employed in previous studies (Rapp et al., 1964a, b, 1965); the virus has been serially passed several times in African green monkey kidney cultures (GMK). The origin and passage history of the adenovirus-SV40 hybrid (strain LL, stock SP2, supplied by B. A. Rubin, Wyeth Laboratories) has been documented (Huebner et al., 1964; Rowe and Baum, 1964; Rapp et al., 1964c). The original SP2 was passed two additional times in GMK cells prior to this study.

Stocks of virus were prepared in the appropriate cell type growing in 16-oz (454 g) bottles. When 50 to 75% of the cells exhibited cytopathic effects (CPE), the cells were disrupted by two alternate cycles of quick-freezing and thawing. The resulting suspension was clarified by low-speed centrifugation. The fluids were dispensed into am-
poules, which were then sealed; the virus was quick-frozen, and was stored at −0.5 C. Assays of the virus were carried out as described below.

In addition, the adeno-virus-SV40 hybrid was checked for ability to induce SV40 tumor antigen in GMK cells; a careful check was also carried out on each harvest for ability to induce SV40 virus antigen as a screen for possible contamination by SV40. None was detected.

Tissue cultures. GMK cells were grown in 16-oz or 1-oz bottles (28.35 g) containing 10% fetal calf serum. GMK cells were grown in 16-oz or 1-oz (28.35 g) bottles previously described (Melnick et al., 1964b). The primary cultures were trypsinized and grown on 15-mm cover glasses in plastic petri dishes in 5% CO2 at 37 C (Rapp et al., 1964a). Nutrient fluids contained Melnick-Hanks Medium (MH) and 2% calf serum; and 1% agar was added to the petri dishes. The plates were incubated at 37 C in a 5% CO2 incubator for 8 days, when a second inoculation was considered a second overlay containing neutral red (1:20,000) was added. Plaques were clearly visible and were counted 1 day after addition of the second overlay.

Mixed adeno-virus and SV40 harvests were plated directly on GMK cells, since SV40 did not plaque on these cells and did not interfere with the titration. When SV40 titers were desired from mixed harvests, the adeno-virus was inactivated by heating the mixture for 1 hr at 50 C (Yang and Melnick, 1963).

Immunofluorescence techniques. The procedures used for the staining technique have been described (Melnick et al., 1964a). The staining procedures used for the detection of SV40 tumor antigen have also been previously reported (Rapp et al., 1964a). Infected cells were treated with sera from hamsters bearing SV40-induced tumors and fluorescein-labeled anti-hamster globulin.

All tests included uninfected cells that were tested with positive serum (and found not to react) and infected cells treated with the homologous antibodies (and found positive). Preparations were examined with a Zeiss fluorescence microscope equipped with a dark-field condenser; an Osram HBO 200 mercury arc vapor lamp was used for illumination.

Chemicals. CA was kindly supplied by The Upjohn Co., Kalamazoo, Mich. Deoxyctydine triphosphate (dCTP) and 2-deoxyctydine (dC) were obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio. The chemicals were dissolved in Eagle’s basal medium, 1 mg per ml, and were diluted directly into the maintenance fluid to give the final concentration desired.

RESULTS

Effect of prior inoculation with SV40 virus. Enhancement experiments were carried out in 1-oz bottles of primary GMK cells. The cultures were inoculated with SV40 at an input multiplicity of approximately 1 plaque-forming unit (PFU) per cell; 24 hr later, the supernatant fluid was removed, and the cultures were inoculated with the nonintegrated adeno-virus or the adeno-virus-SV40 hybrid population, also at a multiplicity of about 1 PFU (titrated in HEK) per GMK cell. Cultures inoculated only with the superinfecting virus, and unoinoculated controls, were included with each test. Unadsorbed virus was removed by washing twice with tris(hydroxymethyl)aminomethane-NaCl after 1 hr of adsorption at room temperature. Cells were harvested 72 hr after the second inoculation; intracellular virus was collected by freezing and thawing the cells without removing the extracellular fluids, as they contained relatively small amounts of virus. The total adeno-virus yields were then titrated in HEK cells, and SV40 virus was titrated in GMK cells.

Prior inoculation of GMK cells with SV40 resulted in a 100-fold increase of adeno-virus 7 over harvests obtained in the absence of SV40 (Table 1). Yields of SV40 virus were neither increased nor decreased. In addition, development of adeno-virus CPE was more rapid in the SV40-inoculated cultures, and a larger percentage of the GMK cells synthesized adeno-virus antigens when comparable experiments were carried out on

<table>
<thead>
<tr>
<th>Initial Adeno- Virus SV40</th>
<th>Superinfec- ting Virus SV40</th>
<th>Loga titer (PFU/ culture) SV40†</th>
<th>Adeno- virus antigen‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adeno-virus 7</td>
<td>SV40</td>
<td>7.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Adeno-virus 7</td>
<td>SV40</td>
<td>5.8</td>
<td>Not done</td>
</tr>
<tr>
<td>None</td>
<td>Adeno-virus 7</td>
<td>&lt;1.0</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* Assayed on HEK cells.
† Assayed on GMK cells.
‡ Adeno-virus antigen detected by immunofluorescence technique; numbers represent approximate percentages of cells containing adeno-virus antigen.
coverglass cultures (Table 1). This antigen was readily detected in groups of cells 24 hr after superinfection of the culture with adenovirus 7 (Fig. 1); at this time, clusters of round cells containing adenovirus antigen were present (Fig. 1). Addition of SV40 24 hr after inoculation with adenovirus 7 did not enhance replication of the adenovirus (Table 1).

A plaque isolate from the adenovirus-SV40 hybrid population was obtained from HEK cells. This isolate failed to induce the synthesis of tumor antigen. Enhancement experiments on GMK cultures similar to those shown in Table 1 resulted in a 100-fold increase in the virus yield. Plaque isolates that were obtained from GMK cells and did induce tumor-antigen synthesis replicated to the same extent in GMK cultures in the presence and absence of SV40. These results are similar to those shown in Tables 1 and 2.

Similar experiments, carried out with the adenovirus-SV40 hybrid population, are summarized in Table 2. Prior inoculation of the cultures with SV40 did not stimulate replication of the hybrid virus. The number of cells synthesizing adenovirus antigen was comparable in the presence and absence of SV40.

Table 2. Effect of prior inoculation of monkey kidney cell cultures with SV40 on replication of adenovirus-SV40 hybrid population

<table>
<thead>
<tr>
<th>Initial virus</th>
<th>Superinfecting virus</th>
<th>Loga titer (PFU/culture)</th>
<th>Adeno-virus*</th>
<th>SV40†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1</td>
<td></td>
<td></td>
<td>Adeno-virus*</td>
<td>SV40†</td>
</tr>
<tr>
<td>SV40</td>
<td>Adenovirus-SV40 hybrid</td>
<td>7.4</td>
<td>5.5</td>
<td>10</td>
</tr>
<tr>
<td>None</td>
<td>Adenovirus-SV40 hybrid</td>
<td>&lt;1.0</td>
<td>5.6</td>
<td>0</td>
</tr>
<tr>
<td>Expt 2</td>
<td></td>
<td></td>
<td>Adeno-virus*</td>
<td>SV40†</td>
</tr>
<tr>
<td>SV40</td>
<td>Adenovirus-SV40 hybrid</td>
<td>6.5</td>
<td>Not done</td>
<td>10</td>
</tr>
<tr>
<td>None</td>
<td>Adenovirus-SV40 hybrid</td>
<td>&lt;1.0</td>
<td>Not done</td>
<td>0</td>
</tr>
</tbody>
</table>

* Assayed on HEK cells.
† Assayed on GMK cells.
‡ Adenovirus antigen detected by immunofluorescence technique; numbers represent approximate percentages of cells containing adenovirus antigen.
ence and absence of SV40, and, again, adenovirus antigen could be readily detected 24 hr after inoculation of the hybrid virus in clusters of cells exhibiting adenovirus CPE (Fig. 2). As indicated in experiment 1 (Table 2), superinfection of the cultures with the hybrid virus population did not enhance replication of the papovavirus.

**Effect of CA.** At 10 μg per ml, CA suppressed the replication of the adenovirus-SV40 hybrid population in GMK cells (Table 3). The harvests from such cultures were unable to induce synthesis of tumor antigen in GMK cells, again indicating the absence of infectious virus. However, virus harvested 72 hr after inoculation of drug-free cultures titered 10^2.5 PFU per culture. The yields of nonintegrated adenovirus were not affected by the concentration of CA used (Table 3).

The effect of CA on the enhancement of adenovirus replication by SV40 was then studied. Results of a representative experiment are summarized in Table 4. Enhancement of adenovirus replication was inhibited by 10 μg of CA per ml. This inhibition occurred when the cells were exposed to CA with the superinfecting virus, or when the antagonist was added with SV40, and the cultures were maintained in CA until the time the cells were harvested. The levels of adenovirus recovered (10^2.3 and 10^4.7 PFU/culture) were comparable to those observed in other experiments (Table 3) when GMK cultures were infected with nonintegrated adenovirus in the absence of SV40. Inhibition of SV40 replication by CA was also marked; no detectable virus was recovered in the presence of CA. The effect of the CA was completely reversed by 50 μg per ml of either CdR or dCTP. Enhancement of adenovirus replication then proceeded normally, and the development of SV40 was similar to that in the absence of CA (Table 4).

**DISCUSSION**

The results of this study confirm and extend the previous studies of O'Connor et al. (1963) and Rabinson et al. (1964) concerning the enhancement of adenovirus replication in monkey cells by SV40. We found that prior infection of monkey cells with SV40 enhances the replication of nonintegrated adenovirus type 7 to about the same extent already reported for adenoviruses 5 and 12. Our previous studies clearly demonstrated that synthesis of tumor antigen is not inhibited by CA, either when induced by SV40 alone (Rapp et al., 1965a, b) or by the adenovirus-SV40 hybrid population (Melnick and Rapp, in press). Therefore, the inhibition by CA of the enhancement of adenovirus replication by SV40 suggests that enzymes involved in the synthesis of SV40 tumor antigen, or tumor antigen itself, are not responsible for the stimulation observed.

Failure of SV40 to enhance replication of the adenovirus-SV40 hybrid population suggests that the virus population studied contains many particles carrying SV40 information. The presence of a large excess of nonintegrated adenovirus should have been reflected by some enhancement of virus yields by SV40. The results with CA point in the same direction. Nonintegrated adenovirus 7 in the hybrid population should have persisted or replicated to some degree in the presence of CA. Failure to detect such virus mitigates against their presence in large excess. The ability of CA to inhibit replication of the adenovirus-SV40 hybrid in GMK cells is evidence that the hybrid popula-

---

**Table 3. Effect of cytosome arabinoside on replication of adenovirus-SV40 hybrid population in monkey-kidney cells**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Compound</th>
<th>Log_{10} titer (PFU/culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus-SV40 hybrid</td>
<td>None</td>
<td>7.5</td>
</tr>
<tr>
<td>Adenovirus-SV40 hybrid</td>
<td>CA*</td>
<td>&lt;1.0†</td>
</tr>
<tr>
<td>Adenovirus 7</td>
<td>None</td>
<td>5.4</td>
</tr>
<tr>
<td>Adenovirus 7</td>
<td>CA</td>
<td>5.0</td>
</tr>
</tbody>
</table>

* Cytosine arabinoside used at 10 μg per ml.
† This harvest was also negative when tested for ability to induce SV40 tumor antigen in GMK cells.

**Table 4. Effect of cytosome arabinoside (CA) on enhancement of adenovirus 7 replication by SV40**

<table>
<thead>
<tr>
<th>Initial virus</th>
<th>Superinfecting virus</th>
<th>Added with initial virus</th>
<th>Added with superinfecting virus</th>
<th>Log_{10} titer (PFU/culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adenovirus*</td>
<td>SV40</td>
</tr>
<tr>
<td>SV40</td>
<td>Adenovirus 7</td>
<td>None</td>
<td>None</td>
<td>7.4</td>
</tr>
<tr>
<td>SV40</td>
<td>Adenovirus 7</td>
<td>None</td>
<td>CA</td>
<td>4.7</td>
</tr>
<tr>
<td>SV40</td>
<td>Adenovirus 7</td>
<td>CA</td>
<td>CA</td>
<td>4.3</td>
</tr>
<tr>
<td>SV40</td>
<td>Adenovirus 7</td>
<td>CA and CdR</td>
<td>CA and dCTP</td>
<td>7.5</td>
</tr>
<tr>
<td>SV40</td>
<td>Adenovirus 7</td>
<td>CA</td>
<td>CA and dCTP</td>
<td>7.3</td>
</tr>
</tbody>
</table>

* Assayed on HEK cells.
† Assayed on GMK cells.
tion reacts to CA like SV40, rather than like non-integrated adenoviruses (see also Buthala, 1964). These results suggest that the incorporation of the determinant of the tumor antigen of SV40 into the virion may control more than the induction of tumor antigen.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant CA-04600 from the National Cancer Institute, and by Public Health Service grants AI 05382, 2 TI-A1 74, and AI 05308 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED


