Origin of Thymidine Kinase in Adenovirus-Infected Human Cell Lines

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Received for publication 12 January 1970

Human adenovirus type 5 enhances the thymidine kinase activity of KB cells but does not induce the enzyme in kinase-deficient HeLa (BU25) cells. Vaccinia induces thymidine kinase activity in both KB and HeLa (BU25) cells. Human adenovirus types 2, 4, 7, and 12 also fail to induce the enzyme in HeLa (BU25) cells. Vaccinia replicates equally well in the presence or absence of HATG (hypoxanthine-aminopterin-thymidine-glycine) in KB and HeLa (BU25) cells. Adenovirus type 5 replicates in KB and in HeLa (BU25) cells in the absence of HATG, and adenovirus type 5 replicates in kinase-positive KB cells in the presence of HATG. However, replication of adenovirus type 5 is grossly inhibited in HeLa (BU25) cells in the presence of HATG. These results suggest that human adenoviruses do not code for a new virus-specific thymidine kinase.

Thymidine kinase (dTK) activity is induced in either thymidine kinase-positive (dTK+) or thymidine kinase-deficient (dTK−) cell lines infected with vaccinia or herpes type viruses (3, 5–8, 14, 15). The dTK activity is also enhanced in polyoma virus-infected dTK+ cell lines, but not in dTK− cell lines productively or abortively infected by the virus (1, 22). An enhancement of dTK activity has been observed after productive infection by simian adenoviruses of African green monkey kidney cells (16, 18), and after either productive or abortive infections by human adenoviruses of human, hamster, and monkey cell lines (2, 11, 18, 20, 21, 23–25). All of the cell lines utilized in the adenovirus studies have been dTK+. The present study was undertaken to learn whether dTK activity is induced by human adenovirus(es) during productive infection of dTK− cells. Enzyme activity was therefore measured at various times after infection of kinase-deficient HeLa (BU25) cells (15) by human adenovirus strains. In addition, the replication of adenovirus type 5 in HeLa (BU25) cells grown in media with or without HATG (see below) was measured. Since aminopterin blocks de novo thymidylate synthesis, adenovirus type 5 would be expected to replicate in the presence of HATG only if the virus induces dTK activity in the HeLa (BU25) cells. As controls, enzyme induction by vaccinia virus and replication of vaccinia in the presence and absence of HATG in HeLa (BU25) cells were studied.

MATERIALS AND METHODS

Cell lines. HeLa (BU25), a subline derived from HeLa S3, is resistant to growth inhibition by bromodeoxyuridine and deficient in dTK activity (15). Cells were grown in Eagles MEM (Auto POW, Flow Laboratories, Rockville, Md.) supplemented with 10% calf serum and subcultured at weekly intervals. KB cells (9) were grown in the same medium but subcultured at 3- to 5-day intervals.

Virus strains. Human adenovirus type 5 (strain adenoid 75) was obtained from American Type Culture Collection. Virus stocks were prepared in either primary human embryonic kidney or KB monolayer cultures in MEM with 5% fetal calf serum and 1 mM L-arginine. Cultures were incubated 72 to 96 hr until cell destruction was complete. The infected cell pellets were resuspended in one-tenth the original volume of phosphate-buffered saline (PBS) and sonically treated for 5 min at 10 kc at 4 C in a Raytheon sonic oscillator. Cell debris was removed by low-speed centrifugation. To the supernatant fluid, 0.02% sterile bovine serum albumin was added. Adenovirus type 5 was titrated by plaque assay on 2-day-old KB monolayers. The overlay medium consisted of Eagle Minimal Essential Medium (MEM) with 10% fetal calf serum, 1 mM L-arginine and 1% agar (Difco). A second overlay containing neutral red was added 6 days postinfection (PI), and plaques were counted from 8 to 14 days PI.

Vaccinia (IHD) clone 4 (5) was propagated and assayed on CV-1 monolayers. The overlay medium for the plaque assay consisted of MEM containing 2% fetal calf serum and 1% agar. A second overlay containing neutral red was added at 3 days PI, and plaques were counted 4 to 6 days PI.

Preparation of enzyme extracts. Confluent monolayer cultures of KB and HeLa (BU25) cells were...
used 3 to 4 days and 6 to 7 days, respectively, after subculture. The cultures were rinsed with isotonic saline solution (GKN) and were infected at multiplicities of 13 to 360 plaque-forming units (PFU) per cell for adenovirus and 8 to 13 PFU per cell for vaccinia. After adsorption for 1 hr at 37°C, unadsorbed virus was removed and MEM containing 10% fetal calf serum and 1 mM L-arginine was added. Cell extracts were prepared 6 to 75 hr after infection with adenovirus and 5 to 8 hr after infection with vaccinia (13). The dTK was assayed as previously described (13) with 3H-deoxyuridine as nucleoside acceptor.

**Virus growth.** Monolayer cultures in 2-oz (60 ml) prescription bottles of 3- to 4-day-old KB or 6- to 7-day-old HeLa (BU25) cells were rinsed with GKN and infected with 0.1 ml of adenovirus or vaccinia at a multiplicity of 0.05 PFU per cell. After 1 hr of incubation at 37°C, the unadsorbed virus was removed and the cultures were rinsed three times with GKN. A 5-ml amount of MEM containing 10% fetal calf serum (1 mM L-arginine was added in adenovirus experiments) was added to half of the cultures. To the rest of the cultures, 5 ml of the same medium was added containing HATG (10−4 M aminopterin, 10−4 M glycine, 10−4 M hypoxanthine, 4 × 10−4 M thymidine; 17, 22). The cultures were incubated at 37°C in a CO2 incubator for the indicated times and then frozen (Fig. 2 and 3). The cells and medium were harvested, and cells were disrupted by sonic treatment at 100 kc at 4°C in a Raytheon sonic oscillator for 3 min for adenovirus type 5 and for 2 min for vaccinia-infected cells. Lysates of adenovirus-infected cells were assayed on KB monolayers, and those from vaccinia-infected cells were assayed on CV-1 monolayers.

**RESULTS**

**dTK activity of virus-infected KB cells.** The effects of vaccinia and adenovirus type 5 infections on the dTK activity of kinase-positive KB cells are shown in Fig. 1. It may be seen that vaccinia enhanced the dTK activity of KB cells two- to threefold at 6 hr after infection. The dTK activity of adenovirus type 5-infected KB cells was enhanced two- to fourfold from 16 to 46 hr after infection. These results confirm previous findings (2, 5, 11, 16, 18–21, 23–25) that vaccinia and adenoviruses enhance the dTK activity of dTK+ cell lines.

**dTK activity of virus-infected HeLa (BU25) cells.** HeLa (BU25) cells are deficient in dTK activity (15). The enzyme activities shown in Table 1 for uninfected cells are minimal, and the variations that occur 7 to 72 hr after mock infection are insignificant. Vaccinia virus infection induces approximately 6.6 units of dTK activity in the kinase-deficient HeLa (BU25) cells (Table 1). This level of induction is about the same as that observed at 6 hr after vaccinia infection of KB cells (11 to 4.5 equals 6.5 units; Fig. 1). In contrast, adenovirus type 5 does not induce a significant increase in dTK activity in HeLa (BU25) cells at any time from 7 to 72 hr after infection (Table 1). In similar experiments, human adenovirus types 2, 4, 7, and 12 failed to induce dTK activity in HeLa (BU25) cells from 1 to 50 hr after infection.

**Vaccinia virus replication in the presence or absence of HATG.** One-step growth curves depicting the replication of vaccinia virus in kinase-positive KB and in kinase-deficient HeLa (BU25) cells in the presence or absence of HATG medium are shown in Fig. 2. Aminopterin-containing HATG medium was employed to inhibit dihydrofolate reductase activity, thereby blocking de novo synthesis of thymidyl acid. In the presence of aminopterin, deoxyribonucleic acid (DNA) synthesis can take place only if endogenous thymidine or the thymidine supplied in the HATG medium is utilized. For the utilization of thymidine, thymidine kinase activity is needed. In vaccinia-infected KB cells, both the cellular and the vaccinia-induced thymidine kinases are available. HeLa (BU25) cells are deficient in dTK activity, but the enzyme induced by vaccinia catalyzes the phosphorylation of thymidine. Thus, replication of vaccinia virus proceeds equally well in either KB or HeLa (BU25) cells in the presence or absence of HATG medium (Fig. 2).
TABLE 1. Thymidine kinase (dTK) activity of adenovirus type 5- and vaccinia-infected HeLa (BU25) cells

<table>
<thead>
<tr>
<th>Hr after infection</th>
<th>dTK activitya</th>
<th>Vaccinia-infected</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Noninfected</td>
<td>Adenovirus type 5-infected</td>
</tr>
<tr>
<td>7</td>
<td>0.2b</td>
<td>0.3</td>
</tr>
<tr>
<td>14</td>
<td>0.2</td>
<td>0.2</td>
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<tr>
<td>16</td>
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<td>0.4</td>
</tr>
<tr>
<td>18</td>
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<tr>
<td>21</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>24</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>26</td>
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</tr>
<tr>
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<td>0.3</td>
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</tr>
<tr>
<td>46</td>
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</tr>
<tr>
<td>48</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>72</td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

a Input multiplicity was about 13 plaque-forming units (PFU) per cell for vaccinia and about 300 PFU per cell for adenovirus type 5. In further experiments, induction of thymidine kinase was not observed after HeLa (BU25) cells were infected with adenovirus type 5 at input multiplicities of about 10, 50, or 200 PFU per cell.

b Expressed as nanomoles of deoxyuridine monophosphate formed per microgram of protein in 10 min at 38°C.

Human adenovirus type 5 replication in KB and HeLa (BU25) cells. One-step growth curves depicting the replication of adenovirus type 5 in KB and in HeLa (BU25) cells are shown in Fig. 3. Adenovirus type 5 undergoes a productive infection in both KB and in HeLa (BU25) cells in normal growth medium. In KB cells, where a cellular dTK activity is available, adenovirus type 5 replicates equally well in the presence or absence of HATG. In contrast, replication of adenovirus type 5 is grossly inhibited in the kinase-deficient HeLa (BU25) cells in the presence of aminopterin (Fig. 3). These experiments demonstrate that neither a cellular nor an adenovirus type 5-induced enzyme is present at activity levels needed for utilization of thymidine so as to permit replication of adenovirus type 5 in HATG-treated HeLa (BU25) cells.

DISCUSSION

Vaccinia and herpes-type viruses induce in various kinase-deficient cell lines dTK enzymes which differ in thermal stability and in kinetic and immunological properties from cellular enzymes (8, 13). The enzyme induced by vaccinia also differs from the enzyme induced by herpes simplex virus in LM(TK- cells) (14). The herpes simplex-induced dTK is immunologically distinct from the enzyme induced by pseudorabies virus (3). These observations and the demonstration that vaccinia and herpes simplex mutants defective in dTK-inducing activity can be obtained (5–7) suggest that new virus-specific enzymes are induced in vaccinia- and herpes virus-infected cells. In contrast, polyoma virus enhances the dTK activity of kinase-positive cells but fails to induce the enzyme in kinase-deficient cells (1, 12, 22). The latter findings support the hypothesis that the dTK enhanced by polyoma virus infection is not coded for by a viral gene, but instead, probably reflects the increased synthesis of a cellular enzyme, perhaps modified to some extent by a viral product.

The data presented in this study are similar to those obtained with polyoma virus-infected cells. Human adenoviruses enhance the dTK activity of kinase-positive cell lines. The enzyme from adenovirus-infected cells is somewhat changed in thermal stability and kinetic and
electrophoretic properties from the enzyme from uninfected cells (11, 18, 23). However, human adenovirus strains fail to induce dTK activity in kinase-deficient HeLa (BU25) cells. This also suggests that the enzyme enhanced by adenovirus infection is not coded for by a viral gene. A dTK with altered kinetic and immunological properties is induced in kinase-positive cells by simian virus 40 (4, 10, 12, 17). It remains to be seen whether simian virus 40 can induce this enzyme in kinase-deficient cell lines.

ACKNOWLEDGMENTS
This investigation was aided by grants from the American Cancer Society (E-291F), the National Science Foundation (GB 8469), the Robert A. Welch Foundation (Q-163), and by Public Health Service grants (CA-06656-08, 1-K6-AI-2352, and 5-K3-CA 25,797) from the Institute of Allergy and Infectious Diseases and the National Cancer Institute, respectively. We thank Carolyn Smith for able technical assistance.

LITERATURE CITED


