NOTES

Enhancement of the Replication of Human Adenoviruses in Simian Cells by Simian Adenovirus SV15

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Received for publication 17 April 1967

Previous studies have shown that simian papovavirus 40 (SV40) possesses the ability to enhance the replication of human adenovirus types 1–7, 12, 14, 16, and 21 in African green monkey (GMK) cells (see review by F. Rapp and J. L. Melnick, Progr. Med. Virol. 8:349, 1966). The helper activity of the papovavirus for adenovirus replication was felt to be SV40-specific, since initial attempts to demonstrate enhancement by a number of other viruses failed (L. A. Feldman et al., J. Bacteriol. 91:813, 1966). The results of experiments now presented clearly reveal that simian adenovirus SV15, which replicates in GMK cells, also has the ability to enhance the replication of human adenoviruses in these cells.

GMK cells were grown in 30-ml prescription bottles as previously described (J. L. Melnick et al., p. 194, in E. H. Lennette and N. J. Schmidt [ed.], Diagnostic Procedures for Viral and Rickettsial Diseases, 3rd ed., American Public Health Association, New York, 1964) in Melnick-Hanks’ lactalbumin hydrolysate (M-H) medium containing 2% calf serum. Maintenance medium consisted of M-H without serum.

Adenovirus type 2 was derived from a fresh human isolate and was used after six passages in KB cells. Adenovirus type 7 was also derived from a fresh human isolate and was used after five passages in human embryonic kidney (HEK) cells. The SV15 virus had been plaque-purified three times in GMK cells. None of the virus stocks, nor the viruses from any of the experiments, were able to induce synthesis of SV40 tumor (T) antigen in GMK cells. All virus stocks were shown by electron microscopy techniques to be free from adenovirus associated satellite viruses (H. D. Mayor et al., J. Bacteriol. 90:235, 1965).

Plaque assays for human adenoviruses were carried out in HEK cells grown as monolayers in 35-mm plastic petri dishes. In those experiments involving coinfection with SV15, neutralization of the mixed harvests with SV15 antiserum was necessary prior to the adenovirus titration. The SV15 was assayed in a similar manner on GMK cell monolayers grown in 60-mm plastic petri dishes after the neutralization of the human adenovirus with type-specific antiserum. In both

![Fig. 1. Replication of human adenovirus type 2 in green monkey kidney cells in the absence and presence of simian adenovirus (SV15) added simultaneously, or 6, 12, or 24 hr after the human adenovirus. Adenovirus type 2 was added at a multiplicity of about 3 PFU per cell. SV15 was added at a multiplicity of 1 PFU. The adenovirus was titrated in human embryonic kidney cells after neutralization of SV15 with specific antiserum.](http://jvi.asm.org/)

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Fig. 2. Replication of human adenovirus types 2 and 7 in green monkey kidney cells in the absence and presence of simian adenovirus (SV15) added 18 hr after the human adenoviruses. Adenovirus type 2 was added at a multiplicity of about 3 PFU per cell. Adenovirus type 7 was added at a multiplicity of about 6 PFU per cell. SV15 was added at a multiplicity of about 1 PFU per cell. The adenoviruses were titrated in human embryonic kidney cells after neutralization of SV15 with specific antiserum.

assays, 0.1 ml of virus inoculum was allowed to adsorb for 1 hr at 37 C. The 60-mm plates also received 0.2-ml of tris (hydroxymethyl)aminomethane buffer (pH 7.4) as carrier fluid to facilitate even distribution of the virus. The overlay consisted of Eagle’s basal medium, 10% fetal bovine serum, 1% agar, and 0.23% sodium bicarbonate; 7 days later, a second overlay containing 1:20,000 dilution of neutral red was added. Plaques were counted on the 10th day after inoculation of the cultures. All titrations were carried out by use of two plates per dilution.

Rabbits were used for the preparation of neutralizing antiserum against adenovirus types 2 and 7, and against SV15. The immunizing viruses had been purified by equilibrium centrifugation in gradients of cesium chloride.

The results of experiments in which GMK cells were inoculated with SV15 (1 PFU per cell) either simultaneously or 6, 12, or 24 hr after exposure to human adenovirus type 2 (3 PFU per cell) are summarized in Fig. 1. In the absence of SV15, total (intra- and extracellular) infectious titers of adenovirus 2 reached a low 12 hr after inoculation of the cultures and showed little increase during the 60-hr experimental period. This is in sharp contrast to the amount of adenovirus recovered from cultures co-infected with SV15. Enhancement of adenovirus 2 replication was observed in all doubly infected cultures. When SV15 was added simultaneously or 6 hr after adenovirus infection, a decrease in infectious adenovirus 2 during the first 12 hr was followed by a sharp increase that began prior to 24 hr after the inoculation of the human adenovirus. The titer of adenovirus 2 48 hr after the human adenovirus was added (but 48 and 42 hr after SV15 infection, respectively) was more than 150-fold greater than the corresponding titer from cultures inoculated with adenovirus 2 alone. Addition of SV15 12 and 24 hr after inoculation of the human adenovirus 2 resulted in a longer latent period corresponding to the delay in addition of the SV15. Again, this was followed by a sharp increase in infectious adenovirus.

Similar results were obtained with adenovirus...
type 7. In these experiments, SV15 was added 18 hr after the cultures were inoculated with the human adenoviruses. Adenovirus 2 was included for comparative purposes. The cultures were inoculated with an input multiplicity of about 6 PFU of adenovirus type 7 or about 3 PFU of adenovirus 2 per cell. Input multiplicity of SV15 was 1 PFU per cell. The results of this experiment are graphed in Fig. 2. In cultures inoculated only with the human adenoviruses, titers again remained low for 48 hr after inoculation. The human adenoviruses in cultures co-infected with SV15 again replicated well. The replication cycle of SV15 (Fig. 3) remained unaltered when the virus was added to cultures inoculated 18 hr previously with human adenovirus 2. Similar results were obtained with SV15 and adenovirus 7. Preliminary results have also revealed that oncogenic simian adenovirus SA-7 can enhance replication of human adenoviruses in simian cells.

The human adenoviruses are able to induce the synthesis of adenovirus T antigen (L. A. Feldman et al., J. Bacteriol. 91:813, 1966; R. A. Malmgren et al., J. Bacteriol. 91:262, 1966) and virus-specific deoxyribonucleic acid (F. Rapp et al., J. Bacteriol. 92:931, 1966) during the abortive cycle in the monkey cells. It therefore appears likely that a late step in adenovirus replication is affected.

This suggestion is supported by this study and by previous observations that the replication of the human adenoviruses in simian cells in the presence of SV40, of defective SV40 (W. P. Rowe and S. G. Baum, J. Exptl. Med. 122:955, 1965; J. S. Butel and F. Rapp, J. Bacteriol. 91:278, 1966), and of a monkey cell-adapting defective component (J. S. Butel and F. Rapp, Virology 31:573, 1967) also follows the pattern of replication of the helper virus.

The simultaneous increase in infectious SV15 virus and adenovirus obtained in doubly infected cultures immediately after a 12-hr latent period (after the addition of SV15) also suggests that an event occurring late in the replicative cycle of SV15 is responsible for enhancing adenovirus replication.

The results of these experiments indicate that enhancement of the replication of human adenoviruses in green monkey cells, previously thought to be a property only of genetic information supplied by simian papovavirus 40, is also a property of some of the simian adenoviruses.

This investigation was supported by Public Health Service research grants CA-04600 and CA-10036 from the National Cancer Institute, and research grant AI-05382 and training grant 5 T1 AI 74 from the National Institute of Allergy and Infectious Diseases.