Adenovirus Susceptibility to Human Interferon During One-Step Replication¹

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Susceptibility of adenovirus types 2, 7, and 12 to human interferon was measured in three human diploid cell strains during a single-cycle infection. Although the relative susceptibility of adenovirus to interferon varied in these cell strains, the final yield of each type in each cell strain decreased as the interferon dose increased. On the other hand, wide difference in interferon susceptibility of adenoviruses and vesicular stomatitis virus (VSV) was noted, as interferon doses above 100 units profoundly inhibited VSV but not the adenoviruses.

There is an increasing number of reported observations that some viruses are apparently less susceptible to a given species of interferon than are others (6, 10, 15, 18). Thus, a given dose of interferon will induce a quantitatively greater resistance to one virus than another. This relative susceptibility of viruses to interferon has received little attention. Indeed, most of the models for the mechanism of action of interferon postulate an equal effect on the replication of all viruses. Adenoviruses have, in the past, been generally regarded as not being susceptible to interferon-mediated interferon (2, 3, 9, 12, 19). However, all of the 12 adenovirus strains we tested were susceptible to interferons by a plaque reduction method, although to a lesser degree than are most other virus groups (7, 8). These data seemed to suggest that this may be an intrinsic property of adenoviruses since limited antiviral activity was demonstrated in more than one cell strain and with more than one species of interferon (7). Whether or not this property of adenoviruses might also be shown by other testing methods was not investigated. The present studies were, therefore, undertaken to investigate the adenovirus susceptibility to interferon in three different human cell strains under single-cycle growth conditions. The results presented confirm and extend the information on the relatively limited susceptibility of adenoviruses to human interferon.

MATERIALS AND METHODS

Interferon preparation. Interferon was obtained from cultured human embryo fibroblasts (HEF) inoculated

with low multiplicities of the Barnes strain of mumps virus by a method described previously (7). Human interferon preparations were titrated in human embryo kidney (HEK) cell cultures in plaque reduction assays with challenge doses of 200 plaque-forming units (PFU) of vesicular stomatitis virus (VSV, Indiana strain). Titers were expressed as the reciprocal of the highest interferon dilution which in interferon-treated cultures reduced by 50% the number of plaques formed in control cultures.

Cells and viruses. The human foreskin fibroblast (HFF) cultures were grown in minimal essential medium supplemented with 10% fetal bovine serum. Other cell culture procedures, adenovirus propagation, and plaque assay methods have been described in detail elsewhere (7, 8).

Yield-inhibition assays. Inhibition of adenovirus yields in the presence of interferon was measured in three different human cell strains: HEK, HEF, and HFF. Monolayers of these cultures were prepared in 60-mm petri dishes in a humidified 5% CO₂-air atmosphere. Cultures were treated with 4-ml volumes of twofold dilutions of human interferon or control fluid (spent tissue culture medium) for 16 hr at 37 C. After interferon or control fluids were removed, the cultures were washed three times with Hanks balanced salt solution. Virus infection was initiated by inoculating plates with 0.2-ml samples of an appropriate dilution of stock adenovirus. After 4 hr of adsorption at 37 C, the monolayers were again washed before receiving 5 ml of maintenance medium, followed by incubation at 37 C. Adsorbed multiplicities were determined by measuring the difference in plaque titer of the inoculum before and after exposure to cells. After incubation for one complete cycle of virus multiplication (28-36 hr), the cells were scraped into the medium and then disrupted six times by freezing and thawing. Infectivity assays (PFU/ml) of pooled adenovirus yields were performed in HEp-2 cell cultures at 37 C. VSV was adsorbed for 1 hr at 37 C, and only two cycles of freezing and thawing were

¹ Some aspects of this investigation were presented at the 70th Annual Meeting of the American Society for Microbiology, Boston, Mass., 26 April 1970.

required to release cell-associated virus. VSV yields reached their maximum at approximately 12 hr. Pooled VSV yields were assayed (PFU/ml) in HEP-2 cultures. $\log_{10}$ inhibition of virus yield was calculated by comparing the yield from interferon-treated cultures with the yield from control cultures.

RESULTS

Reductions of adenovirus yields after single-cycle growth in HEK cultures treated with human interferon are shown in Fig. 1. All three adenovirus types showed significant inhibition at interferon doses above 500 units, with adenovirus type 2 showing marked inhibition (80–96%) at all interferon levels. Significant inhibition in yield was defined as $0.5 \log_{10}$ (70%) or greater reduction from control yields after approximately one cycle of growth (1). The data from these experiments correlate well with that of plaque reduction experiments conducted previously in HEK cultures (7). In all plaque reduction experiments, significant reduction (50% and above) was noted at and above a dose of 125 units of interferon; in yield-inhibition experiments, significant reduction (70% and above) was usually approximated in HEK cultures at and above a dose of 500 units

ADENOVIRUS YIELD INHIBITION IN HEK CELL CULTURES TREATED WITH HUMAN INTERFERON

FIG. 1. Adenovirus yield-inhibition in HEK (human embryonic kidney) cell cultures. Cultures were treated with interferon for 16 hr before challenge with an adenovirus at an adsorbed multiplicity of 10 PFU/cell. Thirty-six hours after virus adsorption, cultures were harvested and infectivity titers were determined by plaque assay in HEP-2 cell cultures. Significant inhibition is defined as $0.5 \log_{10}$ (70%) or greater reduction. Total yield (PFU) of progeny virus from control cultures was 2.2×10^9 for adenovirus type 2, 2.5×10^9 for adenovirus type 7, and 7.8×10^8 for adenovirus type 12.

of human interferon. Thus approximately four-fold more interferon is required to reduce adenovirus yields by 70% during one-step growth than is required to diminish by 50% or more adenovirus plaque counts in the same human cell strain. It is true that these two assay systems do not entirely lend themselves to comparison because of marked differences in both time and multiplicity of infection. However, this sort of quantitative decrease in yields would appear to require a higher relative degree of protection than that required in a plaque reduction assay, where a somewhat lower overall degree of protection may still serve to prevent infection of a large proportion of those cells in the culture which do adsorb virus from the low dose of challenge virus. Figure 2 represents the data obtained for adenovirus types 2, 7, and 12 in neonatal HFF cultures. Cells used in these experiments were from the 19th to the 24th passage levels of the strain. Reductions in virus yields exceeded $0.5 \log_{10}$ units for each type at all interferon doses used (125–4,000 units). Figure 3 represents the data from experiments with the cell strain, HEF, used to prepare the human interferon used throughout this work. Although the data from these experiments are not as dramatic as those obtained with HEK and HFF cells, all three adenovirus types showed significant reductions in yields at the highest dose (4,000 units) of interferon, and somewhat similar curves were obtained for all three types. Why

ADENOVIRUS YIELD INHIBITION IN HFF CELL CULTURES TREATED WITH HUMAN INTERFERON

FIG. 2. Inhibition of adenovirus yields in HFF (human neonatal foreskin fibroblast) cell cultures. Experimental procedures were the same as described in Fig. 1. Total adenovirus yield (PFU) from control cultures was 7.5×10^9 for adenovirus type 2, 1.3×10^9 for adenovirus type 7, and 2.8×10^9 for adenovirus type 12.

somewhat lower values for adenovirus yield-inhibition were obtained in HEF as compared with HEK or HFF with the same lot of interferon is not clear, especially since the interferon preparation was titered by plaque reduction in each cell strain and showed a similar degree of protection in each against a VSV challenge. In light of this finding, it was of interest to examine VSV susceptibility to interferon under conditions of single-cycle growth. Under such conditions VSV should be markedly more susceptible to interferon than were the adenoviruses if the relationship seen under multiple-cycle conditions (the plaque reduction assay) had any validity. Figure 4 shows the reductions in VSV yields from interferon-treated HEK cell cultures in one-step growth experiments. In this and each of the other two cell strains, a given dose of interferon produced a more pronounced depression of VSV yields than was obtained for adenovirus yields.

Doses above 100 units virtually effected complete inhibition of VSV multiplication, a finding not yet obtained for any of the adenoviruses with any interferon dose level. Thus, the data from both multiple-cycle and single-cycle experiments with representative adenovirus strains and VSV indicate that adenoviruses are susceptible to interferon, but relatively less so than VSV.

DISCUSSION

The results presented here demonstrate that the adenoviruses tested exhibited a markedly lower susceptibility to human interferon than did VSV in single-cycle yield inhibition experiments. This limited susceptibility was demonstrable in each of

INHIBITION OF VSV YIELDS FROM
HEK CELL CULTURES TREATED WITH INTERFERON

FIG. 4. Inhibition of VSV yields in interferon-treated HEK cell cultures. Cultures were treated with interferon for 16 hr before challenge with VSV at an adsorbed multiplicity of 10 PFU/cell. Twelve hours after virus adsorption, cultures were harvested and infectivity titers were determined by plaque assay in HEp-2 cell cultures. Total yield (PFU/culture) from control cultures was 4.3×10^5 .

three human cell strains and contrasted with the relative susceptibility of VSV to the same interferon in the same cells. Clear slope differences were found between the VSV and adenovirus dose response curves. Inhibition of adenovirus yields did not reach a maximum at the interferon levels employed nor was an interferon-resistant "persistent fraction" seen in adenovirus plaque reduction assays (7). In a previous study using a plaque reduction method in HEK cultures, VSV appeared to be 50 to 100 times as sensitive to human interferon as representative adenovirus types (7). In yield-inhibition experiments in the same cells, VSV was 10 to 20 times as susceptible as adenoviruses. These differences may be attributed to the fact that the multiplicity of infection in yield reduction experiments is at least 1,000-fold greater than that used in the plaque reduction method. Thus, the single-cycle yield inhibition method seems to reduce somewhat the magnitude of the difference in susceptibility between the rhabdovirus and the adenoviruses. An even greater discrepancy between results obtained with multiple- and single-cycle assay methods has recently been reported by Hallum et al. (11). Multiple-cycle assay methods appeared to exaggerate differences in susceptibility to interferon of VSV and Newcastle

ADENOVIRUS YIELD-INHIBITION IN HEF CELL CULTURES TREATED
WITH HUMAN INTERFERON

FIG. 3. Inhibition of adenovirus yields in HEF (human embryo skin and muscle fibroblast) cell cultures. Experimental procedures were the same as described under Fig. 1. Total adenovirus yield from control cultures was 2.7×10^8 for adenovirus type 2, 4.0×10^8 for adenovirus type 7, and 3.8×10^7 for adenovirus type 12.

disease virus (NDV). The authors attributed this finding to the more rapid decay of antiviral activity against NDV rather than VSV. Whether or not similar factors were responsible for the observed differences in susceptibilities to interferon of adenoviruses and VSV is unknown. It is probable that the decay of the antiviral effect against adenoviruses is more rapid than in the case of VSV, since significant resistance against adenoviruses disappeared when infection was initiated 48 hr or more after removal of interferon, whereas resistance against VSV began to decline 72 hr after interferon removal. Still other factors may alter the degree of antiviral effect of interferon. These may include cellular substances such as stimulon (4) and interferon antagonists (5, 13, 16). Viral properties such as interferon-susceptible or interferon-resistant viral messenger ribonucleic acid, (14) as well as properties inherent in the species of interferon which partially determine its antiviral spectrum (17), are also factors to be considered. Until the mechanism by which these factors influence the antiviral effect of interferon is known, it will be difficult to understand why viruses differ in their susceptibility to interferon.

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