# Inhibition of Adenovirus Type 2 by Human and Monkey Interferon (35804)

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Experiments have thus far shown that adenoviruses exhibit little capacity to induce interferon and are thus generally regarded to be relatively insensitive to its antiviral action (1, 2). A possible explanation may be that adenoviruses possess a spectrum of diverse susceptibility to various types of interferon. As such, the interferon (s) that severely inhibit adenoviruses has not as yet been studied. Human adenovirus types 2, 7, and 12 plaque formation were reported to be inhibited to a limited extent by human interferon (3). The inhibition of adenovirus type 2 replication by rabbit interferon was also reported (3). The susceptibility of adenoviruses to interferon thus far, is clearly less than that shown by many viruses as well as other DNA viruses, *i.e.*, vaccinia or herpes simplex virus (4, 5).

The relationship, if any, that interferon plays in the growth characteristics of adenoviruses in various cell cultures is unknown. It has been noted for years that adenoviruses generally multiply best in cells derived from their natural host (6) and express a limited replication in cells from other animals. For example, most human adenoviruses show little multiplication in primary hamster embryo cells (7) or monkey kidney cells (8). It is doubtful that interferon plays a role in these limited types of infections, since measurable amounts of interferon are not observed with adenovirus infections (1).

This study was performed to determine the extent to which monkey interferon would inhibit Ad 2 growth and to confirm the report by Gallagher and Khoobyarian (3) and that Ad 2 was inhibited by human interferon.

The monkey interferon experiments were performed in Vero cell cultures. This cell line of GMK cells was reported to be defective in producing interferon (9), but is apparently sensitive to its action. Vero cells also allow certain strains of adenoviruses to replicate while other GMK cell lines, *e.g.*, BSC-1, CV-1, and primary GMK cells are restrictive for adenovirus replication (10, 11). The findings show that monkey interferon was more effective in inhibiting Ad 2 antigen synthesis and virion formation than was human interferon.

*Materials and Methods. Interferon induction.* The CG strain of Newcastle disease virus (NDV) was used to induce interferon in cultured cells of human embryonic fibroblasts (HEF) and primary African green monkey kidney cells (GMK). The cells were grown in either 4- or 32-oz prescription bottles and infected with approximately 10 pfu/cell. After a 1-hr adsorption period the cell sheet was washed twice with 10 ml of Hanks' balanced salt solution (HBSS), and then overlaid with 15 ml of Eagles minimal essential medium (MEM, 3% newborn calf serum). Interferon fluids were harvested 24 hr later and acidified to pH 2.5 for 48 hr at 4°, then brought back to pH 7.2 with 0.1 *N* NaOH. Fluids were ampouled in 2-ml amounts and frozen at −70°. Both human and monkey interferon activity was nondialyzable, stable at pH 2.5, and host-cell species specific (12).

*Interferon assay.* Interferon and control fluids were assayed by the tube dilution method (13). Four tubes containing confluent monolayers were inoculated with 0.5 ml of 2-fold diluted interferon or control fluids (fluid removed from uninfected cell cultures). After 12 hr, the interferon fluids were decanted and 100 or 4000 pfu of VSV was added to each tube (virus dose dependent upon assay cells). The interferon titers were

TABLE I. Induction of Interferon in Vero, African Green Monkey Kidney (GMK), and Human Embryonic Fibroblasts (HEF) by Newcastle Disease Virus.

| Interferon source | Expt. | Assay cells | | | |
|---|---|---|---|---|---|
| | | Vero | GMK | RK | HEK |
| Vero | 1 | 0[a] | 0 | 0 | ND[b] |
| | 2 | 0 | 0 | 0 | ND |
| | 3 | 0 | 0 | 0 | ND |
| GMK | 1 | 64[c] | 128 | 4 | 32[d] |
| | 2 | 32 | 64 | 2 | 16 |
| HEF | 1 | 8 | ND | 0 | 2560 |
| | 2 | 8 | ND | 2 | 1280 |

[a] Undiluted.

[b] Not determined.

[c] Reciprocal of interferon dilution inhibiting a cytopathic effect in 50% of the culture tubes infected by 100 pfu of VSV.

[d] 4000 pfu of VSV inoculated/tube culture.

expressed as the reciprocal of the dilution inhibiting a CPE response in 50% of the tube cultures at 24 hr post infection (PI) with VSV. Monkey interferon had reciprocal titers ranging from 64 to 128/ml of GMK cell cultures, 32 to 64/ml on Vero cell cultures, 2 to 4/ml on rabbit kidney cell cultures and 16 to 32 on HEK cell cultures. Human interferon pools had titers of 1280 to 2560/ml on HEK, 8 on Vero cells, and 0 to 2 on rabbit kidney cell cultures (see Table I).

*Challenge virus.* The Ad 2 used in this study has been previously described (14) and was free of adenovirus associated viruses (AAV) (14) as determined by electron microscopy and fluorescent antibody staining. The latter method used conjugates prepared specifically against purified AAV types 1, 2, and 3. Pools of Ad 2 were prepared in primary human kidney or HeLa cell cultures.

*Cell cultures.* Primary GMK, human embryonic kidney (HEK) and HEF were obtained from Flow Laboratories and grown in lactalbumin hydrolysate medium (LAH) in HBSS supplemented with minimal essential vitamins, glutamine, 2–10% calf serum, and 0.04–0.12% sodium bicarbonate. HeLa cells, used for assaying Ad 2 titers, were grown in Eagles basal medium (EBM) supplemented with calf serum and sodium bicarbonate in concentrations indicated above. Rabbit kidney cell cultures were prepared by the method of Bodian (15) and grown in LAH medium.

*Results. Defectiveness of interferon induction in Vero cells.* The Vero cell link of GMK cells was selected for study because of its nonrestrictive nature to certain strains of adenoviruses (11), and in particular to the Ad 2 strain used in this study.

As previously observed, reports indicate that Vero cells were defective in producing interferon but were apparently sensitive to its action (9). The data in Table I confirms this result. No interferon activity was detectable in undiluted fluids from NDV infected Vero cells whether tested on Vero, RK, or GMK cell cultures. Vero cells were sensitive to primary GMK induced interferon, although somewhat less than the primary GMK cells.

The titers of human interferon assayed on HEK cell cultures were always 6- to 8-fold higher than the titers of monkey interferon assayed on GMK cell cultures. It is doubtful that the difference in the capacity of VSV to replicate in HEK and GMK cells explains the variation in titers between human and monkey interferon. When assays were performed on HEF cells, 4000 pfu of VSV were added as challenge virus to each tube, while 100 pfu were added to Vero, RK, or GMK assay tube cultures.

The GMK interferon had a definite effect on VSV replication when assayed on HEF cells, with titers of 16 and 32 observed, while human interferon had a smaller effect on VSV growth in Vero cells. Other reports have previously shown that human and monkey interferons cross the species barrier (9). Furthermore, certain viruses have been shown to appear less sensitive to the antiviral activity of interferon in cells pretreated with the heterologous interferon (16), *e.g.*, human or monkey interferon, which appears to be the case here.

*Sensitivity of adenovirus type 2 to human and monkey interferon.* The effect of a single dose of interferon on the growth of Ad 2 was determined. Tube cultures of Vero and HEK cells were pretreated for 12 hr with either 1 ml of monkey (titer 1:128) or human inter-

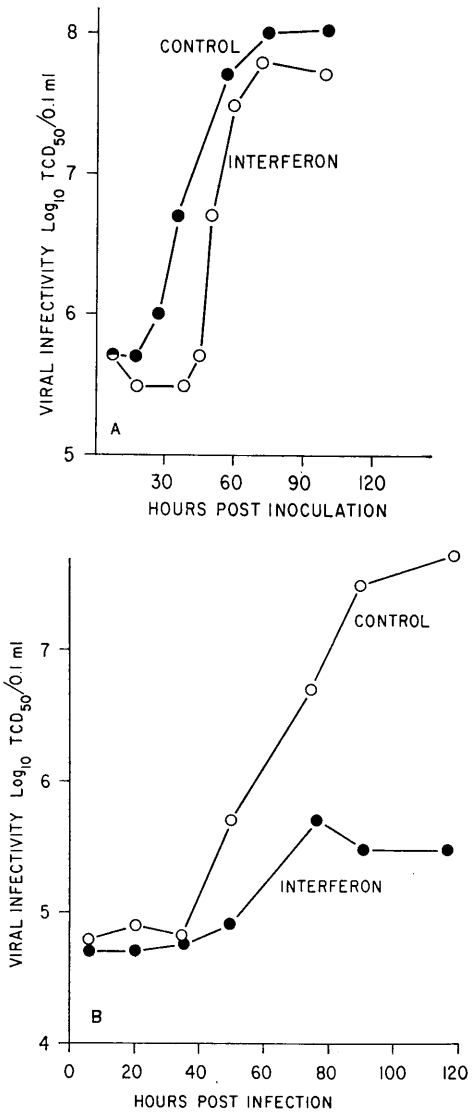


FIG. 1. Inhibition of adenovirus type 2 replication by human and monkey interferon. A comparison of the effect of a single dose of either human (titer 1:1280) or monkey (1:128) interferon on the growth pattern of Ad 2 in HEK (A); and Vero (B) cell cultures. Tube cultures were inoculated with the interferon (contained in 1 ml) 12 hr prior to Ad 2 inoculation. Samples were pooled and removed at the indicated times and analyzed for virus content.

feron (titer 1:1280), respectively. The cell cultures were then infected with Ad 2 (moi, 10 $TCD_{50}$/cell). At various intervals, samples were removed, frozen-thawed 3 times, pooled and assayed for infectious Ad 2. The Ad 2 assays were performed on HeLa cell tube cultures by the 50% end-point method of Reed and Muench (17). The effect of human and monkey interferon on Ad 2 replication is shown in Fig. 1. Monkey interferon reduced Ad 2 yields in Vero cells approximately 95% at 90 hr PI. Human interferon was less effective in reducing Ad 2 yields. A 50–60% reduction in infectious virus was observed at 70 hr PI. Human interferon appeared to have its greatest inhibitory effect earlier in the growth cycle. To investigate this further, tube cultures of HEK were pretreated for 12 hr with 1 ml of 2-fold dilutions of interferon ranging in titer from 160 to 2560. Four tubes of each dilution of interferon, as well as untreated control tubes, were removed at 35 and 70 hr PI. The duplicate samples were pooled and titered for infectious Ad 2. The pattern of inhibition early and late in the growth curve is shown in Fig. 2. At 70 hr PI, high titer interferon fluids were required to show significant inhibition of Ad 2; while, at 30 hr PI, a greater inhibitory effect by interferon treatment was observed. This result may be analogous to the

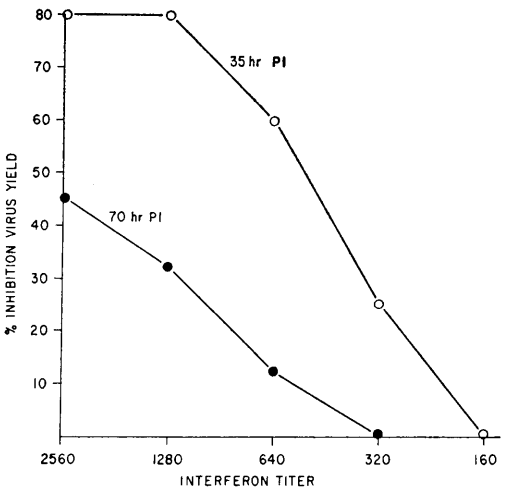


Fig. 2. Inhibition of adenovirus type 2 growth by human interferon. HEK culture tubes were treated with 2-fold dilutions of interferon 12 hr prior to Ad 2 inoculation. At 35 and 70 hr postinoculation the interferon as well as control pretreated cultures were analyzed for virus titer; plotted as a percentage inhibition of control cultures.

TABLE II. Inhibition of Human Adenovirus Type 2 Antigen Formation by Human and Monkey Interferon.

| Virus inoculum (10 $TCD_{50}$/cell) | Cell type | Duration of infection (hr) | Percentage cells fluorescing[a]; cells pretreated with: Interferon[c] Monkey | Interferon[c] Human | Control[d] GMK | Control[d] HEK | Percentage inhibition[b]; Interferon Monkey | Interferon Human |
|---|---|---|---|---|---|---|---|---|
| Ad 2 | Vero | 35 | 0.1 | 5.8 | 6.4 | ND[e] | 98 | 9 |
| Ad 2 | Vero | 70 | 1.2 | 27.0 | 29.5 | ND | 96 | 8 |
| Ad 2 | HEF | 45 | ND | 0.2 | ND | 1.0 | ND | 80 |
| Ad 2 | HEK | 30 | 7.3 | 4.6 | 16.0 | 19.2 | 56 | 76 |
| Ad 2 | HEK | 70 | 66.0 | 23.0 | ND | 68.0 | 2 | 65 |

[a] 40-50 fields counted at × 250, 4 coverslips/evaluation.
[b] Values expressed as a percentage of the inhibition of the control fluid pretreated cultures.
[c] 12-hr treatment with 1 ml of NDV induced HEF (titer 1:1280) or GMK (titer 1:128) interferon.
[d] Medium removed from uninfected cell cultures.
[e] Not determined.

delay in plaque production of Ad 2 in HEK cells by interferon as reported earlier (3). This suggests that at least in some cells, the inhibition was overcome late in the replication process.

*Inhibition of Ad 2 capsid antigen formation by human and monkey interferon.* It would be of importance to determine the effect of interferon on Ad 2 replication at the cellular level. That is, did interferon inhibit Ad 2 capsid antigen formation completely in certain cells in the cell sheet or was the inhibition a general phenomenon resulting in the reduction of Ad 2 progeny and capsid antigens in all infected cells? This was studied by the direct fluorescent antibody method using antibody prepared against purified virus and then conjugated with fluorescein isothiocyanate. Coverslip cultures of Vero, HEF, HEK were treated with either human or monkey interferon 12 hr prior to Ad 2 inoculation. At the times indicated, the coverslips were fixed in acetone and stained with the Ad 2 conjugate. The results were presented in Table II. Monkey interferon showed a greater capacity to inhibit Ad 2 antigen formation (96–98% inhibition) in Vero cells than did human interferon in HEK or HEF cells (65–80%). Both types of interferon reduced the number of cells showing positive fluorescence in homologous cell cultures. Human interferon had little effect on Ad 2 growth in Vero cells, although monkey interferon treated HEK cells displayed a 56% inhibition in fluorescent foci at 30 hr PI but similar cultures fixed at 70 hr displayed only a 2% inhibition. Apparently the inhibition by this heterologous interferon was overcome late in the infection.

In addition to reducing the number of cells fluorescing, interferon treated cultures displayed a lower intensity of nuclear fluorescence suggesting an inhibition in viral antigen synthesis in those cells which were still producing virus.

*Discussion.* There is little evidence demonstrating the sensitivity of human adenoviruses to various types of interferon. Some reports indicate that human and rabbit interferon have a definite but limited effect on adenovirus growth (3). This study confirmed the inhibition of Ad 2 by human interferon and also showed a substantial inhibition of Ad 2 by monkey interferon. The growth curve and immunofluorescent assay procedures used in this study detect infectivity yields and viral antigen formation, respectively. The immunofluorescent assay demonstrates the number of cells producing viral capsid antigen. From the intensity of the fluorescence observed, an approximation of the amount of antigen synthesized in comparison to control cultures can be obtained. The results indicate that interferon treatment inhibits the synthesis of Ad 2 capsid antigens completely in certain cells and also reduces

the synthesis of late viral protein(s) in others.

Adenovirus growth can be inhibited in cell culture by other viruses or viral components. Ad 5 fiber antigen (18) and AAV (19) in sufficient concentration have both been reported to inhibit adenovirus replication. The inhibitory mechanism of fiber and AAV was apparently unrelated to interferon induction (20, 21).

The difference between monkey and human interferon on Ad 2 growth may be explained by the overt difference in the multiplication pattern of Ad 2 in Vero cells. The latent period of Ad 2 in Vero cells was 35–38 hr in comparison to the 12–15 hr latent period observed in HEK cells. Lower titers of progeny virus were always observed from Vero cell cultures. These differences in Ad 2 replication may have been sufficient to allow greater expression of the interferon inhibition in Vero cell cultures.

*Summary.* Human adenovirus type 2 was inhibited to a greater extent by monkey interferon than by human interferon in the cell culture systems used. In both assay systems, *i.e.*, infectivity assays and immunofluorescent staining procedures, a single dose of monkey interferon reduced Ad 2 growth in Vero cell cultures 96–98%, while a 65–80 inhibition was observed with human interferon. The effect of interferon on the total population of infected cells was twofold. The number of cells showing positive fluorescence was reduced in addition to a reduction in the amount of capsid antigens in those cells demonstrating positive fluorescence. These studies suggest that at least certain serotypes of human adenoviruses are sensitive to interferon from animals other than their natural host.

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