Sensitivity of Adenovirus Types 1, 3, 4, 5, 8, 11, and 18 to Human Interferon[1] (35395)

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(Introduced by J. E. Kempf)
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Adenoviruses have in the past been generally regarded as being insensitive to interferon-mediated interference (1–5). However, recent work from this laboratory showed that some members of the adenovirus (Ad.) group were susceptible to interferons, although to a clearly lesser extent than are members of most other virus groups (6).

These preliminary findings seemed to suggest that adenoviruses might share a similar limited susceptibility to human interferon. However, in view of the wide differences in biological and biochemical properties exhibited by members of the Ad. group (7), it seemed appropriate to determine whether, in fact, the sensitivity to interferon shown for Ad. Types 2, 7, and 12 might be shared within the group or might perhaps be type- or even strain-specific in some cases. Accordingly, other representative types and strains were tested against human interferon by means of plaque-reduction assays in human embryo kidney (HEK) cell cultures. This report describes the sensitivity to human interferon of seven additional Ad. serotypes, many of which are significant human pathogens.

Materials and Methods. Interferon induction. Interferon was obtained from cultured human embryo fibroblasts inoculated with low multiplicities of attenuated mumps virus (Barnes strain) according to a method described previously (6). Stock tissue culture interferon preparations were titrated in HEK cell cultures using standard plaque assays and 200-pfu challenge doses of vesicular stomatitis virus (VSV, Indiana strain). Titers were expressed as the reciprocal of the highest interferon dilution which reduced by 50% the number of plaques formed in control cultures. Cell culture procedures, agar overlay medium, and virus propagation have been described in detail elsewhere (6).

Challenge viruses. The prototype strain (adenoid 71) of Ad. Type 1 was obtained from Dr. Floyd W. Denny, Jr., Prototype 3 (strain G.B.) was supplied by the NIH Collaborative Research Program. The prototype strain (RI-67) of Ad. Type 4 was obtained from Dr. M. R. Hilleman; a recent clinical isolate (GL-65) and the vaccine strain (CL 68578) of this serotype were obtained from Dr. M. Rosenbaum. The prototype strains (adenoid 75 and Trim) of Ad. Types 5 and 8, respectively, were also obtained from Dr. Rosenbaum. Dr. Herbert Wenner supplied Ad. Type 11. The D.C. strain of Type 18 was obtained from the American Type Culture Collection.

These strains have been passaged numerous times in HEp-2, KB, or human embryonic kidney (HEK) cell cultures to obtain high titer virus stocks. The characteristics of plaque formation by each type were studied, and most virus types were plaque-purified prior to use. The growth characteristics of Ad. Type 8 deserve special mention. This virus differs significantly in both biological and clinical properties from most other adenoviruses (7). While the amount of complement-fixing viral antigen produced during Ad. 8 infection is equal to or greater than

[1] Some aspects of this investigation were presented at the 20th Annual Meeting of the Tissue Culture Association, Detroit, Michigan, 11 June 1969. Supported in part by the American Cancer Society (Illinois Div.).

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that produced by other members of the group, the yield of infectious virus is usually minimal, being at best 100-fold lower than most other types (7). This very low yield of infectious virus, regardless of the cell strain used for propagation, has hampered the biological and biochemical characterization of this virus (7, 8). However, because of its clinical importance in ophthalmology (Ad. 8 produces a highly specific ocular injection, epidemic keratoconjunctivitis), it was decided to include at least one strain (Trim) of Ad. 8 in these studies. After several passages in HEp-2 cells the virus reached a titer of $10^{5.0}$ pfu/ml.

Plaque assays. Adenovirus titers were determined by the plaque assay in HEp-2 cell cultures according to a procedure described previously (6). Plaque reduction assays with human interferon were done in HEK cell cultures according to the same procedure. Neutral red (0.0075%) was added to the cultures 24 hr prior to the expected development of adenovirus plaques. With all the adenoviruses tested, plaque counts usually increased by about 10% from the count on the first day to reach a plateau on day 5.

The human adenoviruses show wide variations in the time required for development of plaques. While plaque formation in HEp-2 cell cultures by Ad. Types 3, 5, and 11 can be visualized after 8 days of incubation, plaque formation by Types 1, 4, and 8 is not seen before 12–14 days of incubation. Ad. 18 plaques in HEp-2 cell cultures were not observed until approximately 21 days after infection. All of the adenovirus types tested produced plaques in HEK cell cultures, and, in these very sensitive cells, plaques appeared approximately 48 hr earlier than in HEp-2 cell cultures infected with the same virus type.

Results. Sensitivity of adenoviruses to human interferon. To examine the sensitivities of adenoviruses to interferon, experiments were carried out in HEK cell cultures, using groups of three to five replicate cultures in 60-mm plastic petri dishes, each treated with 2 ml of 2-fold dilutions of human interferon or control fluid (spent tissue culture medium) for 16 hr at 37° in a humidified 5% CO_2 in air mixture. Cultures were then challenged with approximately 100 pfu of an adenovirus. Figure 1 shows the patterns of interferon-mediated interference seen for Ad. Types 1, 4, 11, and 18. Each adenovirus exhibits a definite if somewhat limited susceptibility to human interferon. Figure 2 shows the results of representative plaque reduction experiments with the prototype strains of Ad. 3, 5, and 8. Here too, each adenovirus showed significant reductions in plaque counts with several doses of interferon. The size, time of appearance, and final number of plaques were each inversely related to the dose of interferon; high doses not only reduced the final number of plaques, but also retarded the appearance of plaques by from one to several days and also resulted in generally smaller plaques than were observed for that particular serotype in control cultures. This phenomenon was also observed in our previous study (6).

The finding of reduced plaque size in the presence of interferon is not unique to the adenovirus–HEK system but has also been described for other systems (9).

While the vaccine strain of Type 4 may be attenuated (10, 11), it apparently exerts its protective effect through a symptomless selective enteric infection (12). Nevertheless, it was considered possible that this strain might possess greater sensitivity to interferon than other, presumably, more virulent strains. However, in numerous plaque reduction experiments with several different lots of human interferon no detectable differences in sensitivity could be demonstrated among the 3 strains of Type 4 tested (Fig. 1).

Ad. Types 5 and 8 appeared to be somewhat more resistant to the effect of human interferon than the other Ad. serotypes examined in this and a previous report (6). These types required approximately 4- to 8-fold more interferon than was required with other serotypes to effect a 50% reduction in plaque formation.

Discussion. This report describes the relative sensitivity to human interferon of seven additional adenovirus serotypes. Thus, approximately one-third of the human adenovirus serotypes have been examined and most

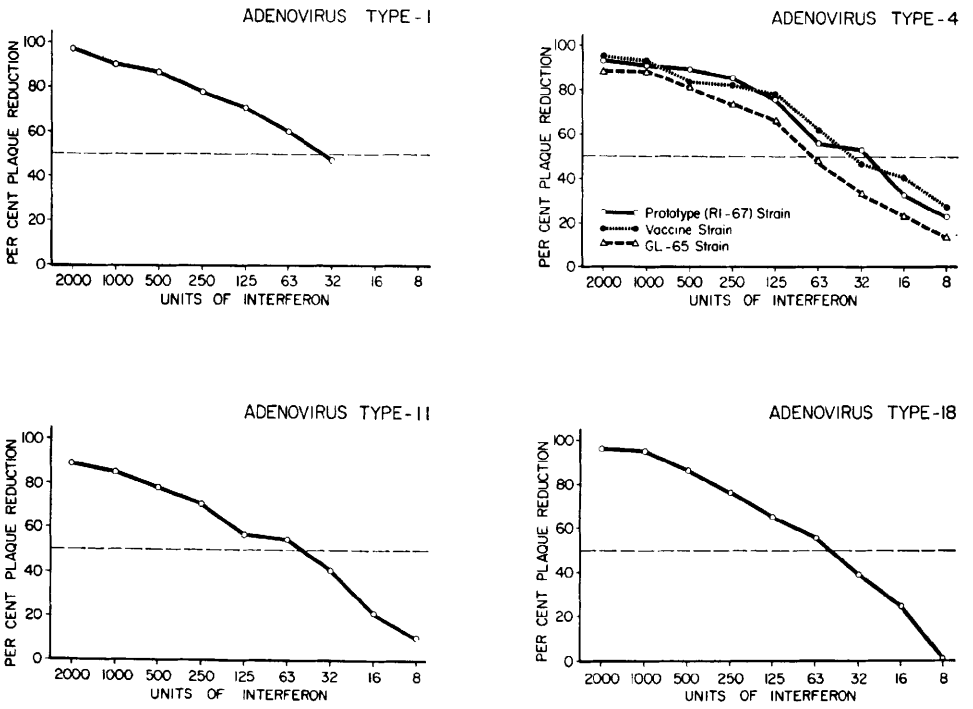

FIG. 1. Sensitivities of adenovirus Types 1,4 (prototype RI-67, wild-type GL-65, and vaccine strain CL 68578), 11, and 18 to human interferon in HEK cell cultures. Three to five cultures were used for each interferon dose. Each culture received a given number of units of interferon contained in 2 ml of maintenance medium. Each adenovirus exhibits a similar low susceptibility to interferon.

have demonstrated a similar, limited sensitivity to the inhibitor. Demonstration of this limited but measurable sensitivity is not confined to one assay method, one cell strain, or even one species of interferon (6, 13). Interferon doses which were sufficient to reduce VSV plaque formation by 50% or more had no effect whatever on adenovirus plaque formation. Doses of human interferon large enough to reduce adenovirus plaque formation by approximately 50% completely eliminated VSV plaque formation.

The subject of relative sensitivity of viruses to interferon is one which has received but scant attention. While it is generally agreed that there are apparent differences in the effect of interferons on different viruses, in most cases it is not at all certain whether the differences are due to inherent properties of the virus, to the species of interferon, or to interferon antagonists produced by the cell strain used in the assay system. Nevertheless, in those reports where different viruses were compared against the same species of interferon by the same assay method, great differences in sensitivity were noted (14–18). Accordingly, it appears that relative viral sensitivity to interferon is a most important variable in the interferon system. Until more is known about the molecular basis for this phenomenon it will be difficult to construct a meaningful model for the mechanism of action of interferon.

There are other interesting, yet unexplained, observations on the relative sensitivities of viruses to interferons. Stewart *et al.* (16) have shown that viruses which are relatively sensitive to an interferon from one animal species may well be resistant to interferons from other species. Thus, in their systems, the order of sensitivity to one interferon within a group of different viruses changed markedly when the same viruses were examined against a variety of different

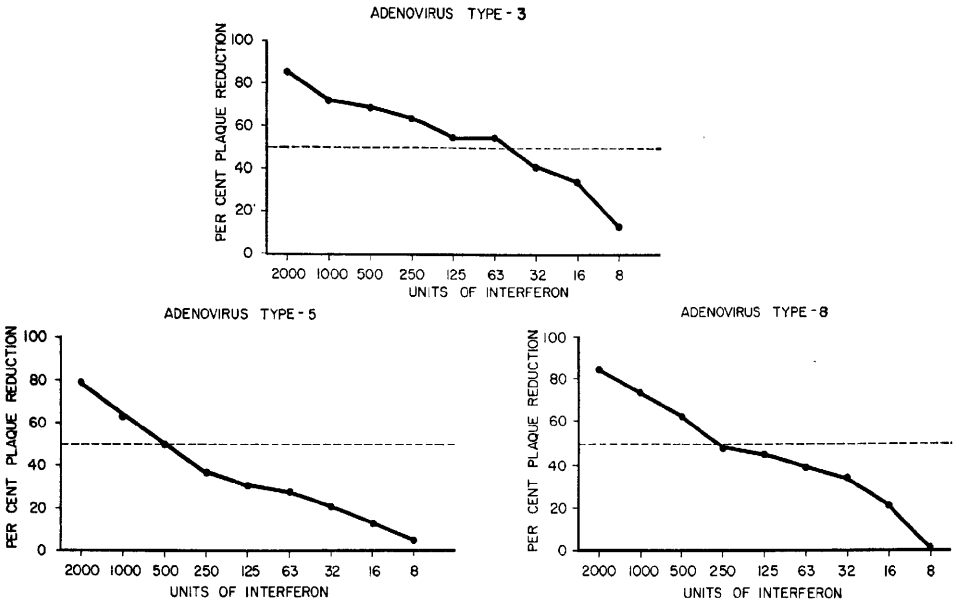

FIG. 2. Interferon sensitivities of adenovirus Types 3,5, and 8 plotted as percentage plaque reduction in interferon-treated HEK cell cultures. Ad. Types 5 and 8 required approximately 4- to 8-fold more interferon to effect significant plaque reduction than did most other Ad. serotypes tested.

species of interferon. The number of critical factors which interact to produce the antiviral effect observed for a given species of interferon in a model system remains unknown but may include such viral properties as "interferon-sensitive" or "interferon-resistant" viral mRNAs (19), cell factors such as "stimulon" (20), "enhancer" (21), and other antagonists of interferon synthesis or action (22, 23), and, finally, properties inherent in the species of interferon which partially determine its characteristic spectrum of antiviral activity (16). Obviously, further work is needed to elucidate the role of these and perhaps other significant variables in interferon systems.

The assay method is also an important consideration. A recent report showed that methods which measure virus yield inhibition under multiple- rather than single-cycle conditions can exaggerate differences in sensitivity to interferon of different viruses (24). This finding was attributed to the more rapid decay of antiviral activity against one rather than the other virus. The amounts of interferon used and the nature of the challenge viruses tested were probably important factors in such experiments. Results in contrast with those of the report cited above were obtained in this laboratory when the sensitivities of VSV and several adenovirus types of human interferon were compared by both plaque reduction and single-cyle yield inhibition methods in HEK cell cultures treated with similar doses of the same lot of interferon. Single-cycle cyields of VSV and adenovirus Types 2, 7, and 12 were obtained within 48 hr of removal of interferon, a time period during which the antiviral effect against any of these viruses did not markedly decay at the interferon levels employed. By both methods, these viruses showed similar relative sensitivities to interferon (13). While direct comparison of yield-inhibition and plaque-reduction experiments is not possible because of the differences in the nature of these assay methods, the fact remains, however, that in our system these two methods compare favorably.

Summary. In a continuing evaluation of adenovirus sensitivity to interferons the limited but measurable sensitivities of Ad. Types

1, 3, 4, 5, 8, 11, and 18 to human tissue culture interferon were demonstrated in plaque-reduction assays using human embryo kidney cell cultures. Generally similar patterns of interferon-mediated interference were observed for each virus type and for several strains within a single serotype. Similar findings have been reported previously for Ad. Types 2, 7, and 12 (6). The relatively limited sensitivity of adenoviruses to interferons seems to set them apart from many other animal viruses and reinforces a number of recent reports of intrinsic differences in sensitivity to interferon among different viruses.

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Received Nov. 4, 1970. P.S.E.B.M., 1971, Vol. 136.