Adenovirus-Associated Viruses: Enhancement by Human Herpesviruses (34919)

NEIL R. BLACKLOW, M. DAVID HOGGAN, AND MITZI S. MCCLANAHAN
(Introduced by R. M. Chanock)

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Laboratory of Infectious Diseases, Bethesda, Maryland 20014

The adenovirus-associated viruses (AAV) are a group of small defective DNA-containing viruses that produce infectious progeny only in cell cultures coinfected with an unrelated helper virus, adenovirus (1–4). This requirement for adenovirus appears to be specific, since infectious progeny are not produced when cultures are coinfected with any of a variety of candidate helper DNA or RNA viruses. This unique association of AAV with adenoviruses is also seen in AAV infections of a natural host, man. Although serologic surveys indicate that man is commonly infected with AAV, these viruses have to date been isolated only from children experiencing adenovirus infections (5, 6). The role of AAV in the initiation or modification of human disease is unknown but the novel concept of human infection with a defective virus has introduced an element of complexity into the determination of the etiology and epidemiology of viral diseases.

A recent observation indicates that either herpes simplex virus or infectious bovine rhinotracheitis virus (a herpes group virus) can contribute a partial helper function(s) to AAV (7). Cell cultures coinfected with AAV and herpes simplex produce AAV-specific immunofluorescent-stainable antigen, but do not produce infectious AAV. Studies have been performed to examine any possible relationship between AAV and other human herpesviruses, EB virus, cytomegalovirus, and varicella-zoster virus; these studies are the subject of this report.

Materials and Methods. Stock pools of AAV type 1(H) and AAV type 3(H) were heated at 56° for 15 min prior to use (2); this procedure eliminates infectious adenovirus without significant inactivation of AAV. Herpes simplex virus (HSV) type 1 was obtained from Dr. J. Rose (NIH), cytomegalovirus (CMV) strain Burch from Dr. W. Rowe (NIH), and herpes zoster virus (HZV) strain Meyer from Dr. L. Martos (Flow Laboratories, Inc.). An EB virus-positive Burkitt tumor cell line (Jiyoye) was obtained from Dr. V. Dunkel (NIH). An EB virus-negative lymphoblastic leukemia cell line (LK1D) and an EB virus-negative Burkitt line (Raji) were provided by Dr. A. Haase (NIH). The strain of adenovirus type 7 (Ad. 7), free of AAV, was used as previously described (2).

HEP-2 and WI38 cell cultures were obtained from Flow Laboratories, Inc. and HEM Research, Inc., respectively. They were trypsinized, grown on coverslips in plastic petri dishes and then used in immunofluorescence (FA) tests by techniques previously described for human embryonic kidney cells (5). Since the AAV do not produce cytopathic effects (CPE) in cell cultures, they are assayed by the detection of AAV-specific antigens [FA and complement-fixing (CF)] and by electron microscopy (EM). The indirect FA method for the quantitative assay of AAV type-specific antigen (8), and the technique for the detection of AAV type-specific CF antigen (2), and EM techniques (2), were performed as previously reported.

HSV, Ad. 7, AAV1, and AAV3 were each inoculated at a multiplicity of 10 $TCID_{50}$/cell; CMV and HZV were each inoculated at a multiplicity of 0.003 $TCID_{50}$/cell. Viruses were adsorbed for 2 hr in maintenance medium and the cells then washed 4 times with fresh medium before final medium renewal. HSV and Ad. 7 infected cells grown on coverslips were fixed in

TABLE I. Helper Effect of Human Herpesviruses and Ad. 7 on AAV1.[a]

Helper virus	Cell culture	AAV1 FA-positive cells (%)	AAV1 CF antigen titer[b]	Presence of AAV by EM	Presence of AAV1 FA on serial passage
Ad. 7	HEP-2	0.5	16	+	+
EB-positive cell line[c]	Jiyoye cell line	2.6	<2	—	—
EB-negative cell line	LK1D cell line	Neg.	<2	—	—
CMV	WI38	0.3	<2	—	—
HZV	WI38	2.2	<2	—	—
HSV	HEP-2	13.4	<2	—	—

[a] AAV1 inoculated in each instance at a multiplicity of 10 $TCID_{50}$/cell. Multiplicity used of each helper virus and time of harvests for FA, CF, and EM as indicated in text. Cells inoculated with each helper virus alone or with AAV1 alone were AAV1 FA negative.

[b] Expressed as reciprocal dilution of antigen.

[c] 6.5% of cells positive for EB virus in both control and AAV1-infected cells.

acetone 24 hr after infection at a time when 25 to 50% of the cell sheet was involved with characteristic CPE. CMV and HZV infected cells on coverslips were fixed 5 days after infection at a time when approximately 25% of the cell sheet was involved with characteristic focal CMV or HZV CPE. At the time of coverslip harvests, fluids from infected cultures were frozen at —70° for later CF, EM, and infectivity tests.

The Jiyoye, LK1D, and Raji tumor cell lines were carried, and FA coverslip preparations of these cell lines were made, according to described techniques (9). Detection of EB virus antigen was by the indirect FA method, using the previously described horse antihuman conjugate (5). For inoculation with AAV, each tumor cell line was washed once with medium RPMI 1629 and then infected with 10 $TCID_{50}$/cell of AAV in a volume of 5 ml of RPMI 1629. After 2 hr, the cells were washed 3 more times and then renewed with RPMI 1629 medium supplemented by 25% heated fetal calf serum. FA coverslip preparations were made and fluids were also frozen at designated time intervals.

Results. The helper effect of human herpesviruses on AAV1 is summarized in Table I. All herpesviruses helped AAV1 to produce FA antigen, but they were unable to elicit either the production of detectable AAV1 CF antigen or physical particles as viewed by EM. By contrast, Ad. 7 helped AAV1 produce not only FA antigen but also CF antigen and AAV particles. It is noteworthy that, under identical experimental conditions, there was a greater percentage of AAV1 FA-positive cells obtained with HSV than with Ad. 7; although not shown in Table I, this finding was also seen in preparations examined from AAV1 FA antigen 48 hr after infection.

Undiluted fluids from cultures coinfected with AAV1 and CMV, AAV1 and HZV, AAV1 and HSV, and AAV1 and Ad. 7 were serially passed in 0.1-ml volumes to fresh cell cultures which were subsequently examined for AA1 FA antigen. As indicated in Table I, AAV1 FA antigen was propagated with Ad. 7 but not with CMV, HZV, or HSV helpers even at the second passage level, indicating that infectious AAV1 was produced only in the presence of Ad. 7.

The Jiyoye and LK1D cell lines were each inoculated with AAV1, and tested in FA 48 hr later. As shown in Table I, AAV1 FA antigen was detected in the EB virus-positive Jiyoye line but not in the EB virus-negative LK1D line. Seven days after exposure to AAV1, each cell line was divided and passed, and then tested in FA 48 hr later. At that time, AAV1 FA antigen was not detected in either cell line. Identical negative findings were noted on 8 subsequent passages, indicating that infectious AAV1 was not produced in either cell line. Although not noted in

Table I, we were unable to produce AAV1 FA antigen in the EB virus-negative Burkitt line, Raji.

The following controls were performed: (a) cells inoculated with each herpesvirus alone or with AAV1 alone did not react with AAV1 antiserum in FA; (b) the helper effect of herpes simplex on AAV1 was also produced on AAV3; these effects were eliminated by prior treatment of herpes simplex with chloroform;[1] (c) the AAV1 staining produced in the presence of herpes simplex or the Jiyoye line was not obtained when AAV1 was reacted with AAV1 antiserum (5) prior to infection; (d) AAV1 antiserum did not react in FA against control uninfected Jiyoye and LK1D lines; further, the horse antiguinea pig conjugate used in the indirect FA tests did not react with the 2 cell lines when tested alone; and (e) all tumor lines were tested for contaminating adenoviruses in CF and FA by previously reported methods (2, 8) and were negative.

Discussion. The data presented here indicate that the human herpesviruses provide incomplete helper function(s) for the defective AAV, as contrasted with adenoviruses which provide all the required functions for a complete helper effect. This finding provides an additional system with which to analyze the sequential events needed for the production of infectious AAV.

The human herpesviruses are associated with a wide spectrum of documented disease, as well as with postulated disease in the case of EB virus. Despite these differences in disease syndromes, the herpesviruses share a common helper relationship with AAV. This helper effect is particularly noteworthy in the case of EB virus, since it shows a biologic activity of this virus that is indistinguishable from that of the other human herpesviruses. To date, human infections with the defective AAV have been closely associated with adenovirus infections in a pediatric population (10). However, the role of AAV in human disease is unknown. Because the human herpesviruses as well as adenoviruses enhance AAV in tissue culture, there is now the possibility that AAV may participate in the disease response to herpesvirus as well as to adenovirus infections.

Summary. Human herpesviruses (EB, cytomegalovirus, herpes zoster, and herpes simplex) provide incomplete helper function(s) for the potentiation of the defective adenovirus-associated viruses (AAV) as contrasted with adenoviruses which provide all the required functions for a complete helper effect. AAV immunofluorescent-stainable antigen is formed in the presence of herpesviruses but infectious AAV is not produced.

[1] Chloroform treatment was as described by Feldman, H. A., and Wang, S. S., Proc. Soc. Exp. Biol. Med. **106**, 736 (1961).

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