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New Human Adenovirus Isolated From a Renal Transplant Recipient: Description and Characterization of Candidate Adenovirus Type 34

JOHN C. HIERHOLZER,* NUZHET O. ATUK, AND JACK M. GWALTNEY, JR.

Center for Disease Control, Atlanta, Georgia 30333, and University of Virginia School of Medicine, Charlottesville, Virginia 22901*

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An antigenically distinct adenovirus is described which was isolated in March 1972 from the urine of a 17-year-old Caucasian male who was experiencing fever after receiving a kidney transplant from a cadaver in February. The adenovirus could not be isolated in April from a pharyngeal swab which yielded cytomegalovirus. Complement-fixation, hemagglutination-inhibition, and/or serum-neutralization tests on sequential serum specimens from the patient confirmed that the adenovirus infection occurred during March and showed that infections with cytomegalovirus and respiratory syncytial virus also occurred during late March and April. The patient's persistent fever, for which other causes could not be found, may have been associated with one or more of these infections. Upper respiratory symptoms and lung involvement were not found during this period. Mild liver dysfunction during this time could not be clearly related to adenovirus infection because of the presence of multiple other causes. The adenovirus may have been latent in the donor kidney and become active in the new host as a consequence of immunological impairment. The adenovirus, purified by terminal dilution and plaque procedures, has antigenic, morphological, biophysical, host susceptibility, and hemagglutinating properties characteristic of adenovirus group 1A. Buoyant densities in CsCl are 1.340 g/ml for the virion, 1.304 g/ml for the group CF antigen (hexon), 1.295 g/ml for the major soluble complete hemagglutinin (dodecon), and 1.206 g/ml for the minor soluble complete hemagglutinin (tentatively, fiber dimer). The virus does not cross-react in reciprocal hemagglutination-inhibition and serum-neutralization tests with antisera to adenovirus types 1 to 33. We propose this virus as candidate adenovirus type 34 (Compton).

Adenoviruses (AV) may frequently be isolated which cannot be definitively typed as one of the recognized human AV serotypes. In many instances one or more of several common difficulties arise to impede further study of the isolate. The isolate may be suspected as being of nonhuman origin, either because it was isolated only in primary monkey kidney tissue culture or because cross-contamination may have occurred from other tissue cultures in the laboratory. Frequently appropriate acute and convalescent serum specimens from the patient are not available, so that infection by the virus cannot be documented by serological means. Another common problem is that original isolation material was not saved for subsequent reisolation attempts, preferably by another laboratory. Or, finally, suitably accurate clinical records may not be available to warrant the extensive laboratory work required to characterize hybrid adenoviruses or totally new antigenic types.

Recently we isolated an "untypable" AV which we studied intensively because of its potential importance in human disease. The virus (Compton strain) was isolated from the urine of a 17-year-old male who had a lengthy episode of unexplained fever after a renal transplant. The virus, which may have been associated with the fever episode, was shown to be a human group I AV with apparently new antigenic characteristics. It is described in this report as candidate AV type 34.

MATERIALS AND METHODS

Clinical summary. The patient (Compton) was a 17-year-old white male admitted to the University of Virginia Hospital on 10 March 1972, for evaluation

and treatment of massive ascites.

In 1967, he presented with subacute glomerulonephritis identified by renal biopsy. He was treated with 6-mercaptopurine and prednisone. While on this treatment in 1969, he developed simultaneous hepatic tuberculosis and generalized histoplasmosis. A liver biopsy showed numerous microgranulomas and acid-fast bacilli compatible with tuberculosis; the diagnosis of histoplasmosis was made by bone marrow biopsy, which showed organisms of characteristic morphology. These infections were successfully treated with appropriate antimicrobial therapy. In 1971, the patient's renal function progressively deteriorated and hemodialysis was initiated. As a result of fluid overload due to excessive salt and water intake, he developed cardiac failure with congestive hepatomegaly and ascites. On 21 February 1972, the patient received a renal transplant from a cadaver. The transplant operation was successful, and serum creatinine levels returned to normal. However, postoperatively and at the time of discharge on 4 March, he had persistent hypervolemia with cardiac failure and moderate ascites. On 10 March, he was readmitted because of increasing ascites. The patient also had hypertension, which was treated with guanethidine, and a seizure disorder, controlled with diphenylhydantoin, which was thought to result from a prior head injury.

When the patient was examined, his height was 178 cm and his weight, 58 kg. He had a pulse rate of 108 beats/min; respiratory rate, 24/min; blood pressure, 170/120 mm Hg; and oral temperature, 37.2 C. The face appeared Cushingnoid. The left border of cardiac dullness was in the mid-axillary line, and a grade III/VI systolic murmur was heard over the area of the pulmonic valve. The abdomen was greatly distended, and shifting dullness and a fluid wave were present. The hematocrit value was 21%, the white cell count was 7,900/mm^3 and the differential showed 2% bands, 86% polymorphonuclear leukocytes, 5% lymphocytes, 3% mononuclear cells, 3% eosinophils, and 1% basophils. Serum chloride was 101 meq/liter; CO_2, 30 meq/liter; potassium 3.5 meq/liter; sodium, 138 meq/liter; blood urea nitrogen, 51 mg/100 ml; glucose, 120 mg/100 ml; creatinine, 1.3 mg/100 ml; calcium, 10.6 mg/100 ml; phosphorus, 2.4 mg/100 ml; uric acid, 9.1 mg/100 ml; total protein, 5.5 g/100 ml; albumin, 3.5 g/100 ml; alkaline phosphatase, 85 IU. Seven blood cultures and three urine cultures grew no bacteria. Cultures of spinal fluid and bone marrow were sterile. VDRL, heterophile antibody, and hepatitis B antigen tests were negative. Chest roentgenograms showed massive cardiomegaly and venous hypertension. Electrocardiogram showed right ventricular and biatrial conduction abnormality, left ventricular hypertrophy, and ST-T changes of ischemia and/or digitalis effect.

The patient was treated with digitalis, diuretics, and salt restriction. On March 24, in the course of a brisk diuresis, he had two grand mal seizures and a brief period of asystole. On March 28, the patient developed fever which persisted with evening spikes from 38 to 38.8 C. A urine specimen obtained on 30 March 1972 grew an adenovirus in human embryonic lung cells (WI-38), which is the subject of this report.

The patient's cardiac condition improved, and he was discharged on April 6, weight 47.3 kg. Because of persistent fever while at home, he was readmitted for evaluation on April 12. Physical examination was unchanged except for absence of ascites. Weight was 45.5 kg and temperature was 39 C. Abdominal roentgenograms showed no change in the size of the transplanted kidney from a previous examination. Nine blood cultures were sterile. Examination of spinal fluid, including microbial cultures, was within normal limits. Urine culture for bacteria was sterile. A tuberculin skin test was negative. The bone marrow was slightly hypocellular. LDH and serum glutamic oxalacetic transaminase were slightly elevated on April 13 and alkaline phosphatase had risen to 226 IU (see Fig. 3). Liver biopsy showed small foci of nonspecific leukocytic infiltration, predominantly in portal areas; no microorganisms or viral inclusions were seen on stained tissue sections. Splenic defects were noted on two consecutive radioisotopic scans; these were thought to represent a splenic infarction or splenic abscess by a radiology consultant.

Culture of pharyngeal secretions collected on April 29 and tested in WI-38 cells yielded a cytomegalovirus, identified by characteristic cytopathic effect. Leukocyte culture in WI-38 cells on April 29 was negative. The patient continued to have daily temperature elevations in the range of 38.5 C. His medications included azathioprine, prednisone, isoniazid, folate, spironolactone, ferrous sulfate, and diphenylhydantoin. Diphenylhydantoin was temporarily discontinued, but fever persisted. He was discharged on May 4 without specific therapy for the fever.

The fever persisted and the patient was readmitted on 15 June 1972 for suspected chronic renal transplant failure. Renal biopsy showed considerable mononuclear cell infiltrates around glomeruli and in the interstitium consistent with rejection phenomenon; viral inclusions were not seen. He was treated with radiation to the transplanted kidney, and a 3-day course of methylprednisolone. The fever disappeared during the methylprednisolone therapy and, although it later reappeared, it was not prominent. He continued to have low-grade temperature elevations until August, but since that time and up to the time of this report, he has not had unexplained fever. After this treatment, creatinine clearance was 33.0 ml/min.

The patient's condition was stable until January 1973, when he was readmitted with an infiltrate in the lower lobe of the right lung. This was thought to be a bacterial pneumonia and was treated successfully with cephalothin. Sputum cultures and smears for mycobacteria were negative.

The patient was readmitted on 10 August 1973 for measurement of plasma and renal vein renin levels because of continuing hypertension. Renin levels were found to be elevated, presumably due to the patient's original kidneys which had not been removed at the time of the transplant. He was readmitted in October 1973, and a bilateral nephrectomy was done. The pathology report showed chronic lobular glomerulonephritis with extensive vascular sclerosis and atrophy. Since the fall of 1973, the patient has felt well and gained solid weight. Blood pressure measurements

have ranged from 100/80 to 130/90 mm Hg on furosemide and methyldopa. He is currently (January 1975) attending a military college and engaging in full activity with normal blood pressure and allograft function (creatinine clearance, 46.6 ml/min).

Virus isolation. Original urine and throat swab (single cotton swab in 5 ml of beef heart infusion broth with 1% bovine serum albumin) specimens were processed independently in two different laboratories at different times. In Laboratory 1 (JMG), the specimens were immediately inoculated onto roller cultures of WI-38 cells as previously described (13).

In Laboratory 2 (JCH), after a 2-year period of storage at −60 C, a 2.25-ml portion from each specimen was mixed with 0.25 ml of 10× treatment medium consisting of tryptose phosphate broth with 0.5% gelatin, 16,000 units of penicillin, and 8,000 μg of streptomycin per ml. After clarification at 1,000 × g at 2 C for 10 min, 0.5 ml of the specimen was inoculated into one tube each of diploid fibroblast (HELF), continuous human epidermoid carcinoma (HEp-2), primary human embryonic kidney (HEK), and primary rhesus monkey kidney (MK) tissue cultures, in that order. The maintenance medium for fibroblast cultures was Eagle minimal essential medium (MEM) with a 2% fetal calf serum and 0.07% bicarbonate; for HEK and HEp-2, MEM with 2% serum and 0.14% bicarbonate; and for MK, MEM with no serum and 0.14% bicarbonate. All maintenance media contained 50 μg of chlortetracycline (Aureomycin) per ml, so that the final antibiotic concentration in inoculated tissue cultures was 533 units of penicillin, 267 μg of streptomycin, and 33 μg of Aureomycin per ml of culture fluid. HELF and MK cultures were rolled; HEp-2 and HEK cultures were stationary. All cultures were passaged and observed for a minimum of 4 weeks for cytopathogenic effects (CPE). In addition, MK cell cultures were washed once with Hanks balanced salt solution (HBSS), covered with 1 ml of HBSS, and hemadsorbed with 0.1 ml of 0.4% guinea pig erythrocytes (RBC) to detect myxoviruses before these cultures were discarded as negative. Isolates recovered were identified by standard hemagglutination (HA) and hemagglutination-inhibition (HI), complement-fixation (CF), and serum-neutralization (SN) tests (4, 23, 24, 47).

Strain purification. The adenovirus was strain purified from an early HEp-2 passage by consecutive triple terminal dilution passages in HEK followed by triple-plaque purifications in HEp-2. Terminal dilutions were carried out by titrating the virus through a 10^{-2} to 10^{-7} dilution series in HBSS, inoculating 0.1 ml of each dilution in each of three HEK tubes, and harvesting the highest dilution showing minimal CPE after 10 to 14 days of incubation at 36 C. The end-point dilution tubes were harvested by three freeze-thaw cycles, pooled, and again passaged as a dilution series in HEK. This process was repeated for a total of three end-point dilutions in HEK.

The virus in the highest dilution showing 2^+ to 3^+ CPE from the last dilution series was diluted through a 10^{-1} to 10^{-4} series and inoculated onto HEp-2 monolayers in Falcon petri dishes (60 by 15 mm) for development of plaques. The procedure for plaque purification was modified from a plaque test for RSV described by Coates et al. (6). HEp-2 cells were trypsinized and planted in the dishes at a concentration of 6×10^5 cells per dish in 10 ml of a growth medium consisting of 90 parts of Eagle MEM, 10 parts of 0.22 μm-filtered but unheated fetal calf serum, and 50 μg of Aureomycin per ml. The plates were held at ambient temperature for 2 h and then incubated at 37 C under a 5% CO_2/70% humidity atmosphere for 1 to 2 days.

The cell sheets (approximately 90% confluent) were washed twice with HBSS and inoculated with 0.2 ml of virus dilution. The inoculum was allowed to adsorb to the cell sheet for 2 h in the CO_2 incubator, with occasional rotation to evenly distribute the virus, and was then removed by aspiration with two more washes with HBSS. The cell sheets were immediately overlayed with 5 ml of a medium consisting of 2.0% sea-plaque agarose (Marine Colloids, Inc., Rockland, Me.) in MEM with 1% fetal calf serum, 50 μg of Aureomycin per ml, 0.14% bicarbonate, and 0.103 mM glutamine (all final concentrations). The cultures were incubated in the CO_2 atmosphere for 7 days. At that time the highest dilution showing isolated plaques was passaged by inoculating plaques, picked with a needle, into HEK to raise the virus concentration for the next end-point plaque series in HEp-2 dishes. The process was repeated two more times. The strain-purified virus was then passaged in 32-oz prescription bottles of HEp-2 to build a working virus stock; hence the urine/$WI\text{-}38_1HEp_1HEK_3$-$(HEp_1HEK_1)_3HEp_2$ passage (strain purified) as well as a urine/$WI\text{-}38_1HEK_1HEp_2$ passage were used throughout this study.

Sterility checks on final stock passages were carried out by inoculation of various bacteriological, mycological, and mycoplasmal media to reveal nonviral contaminants (21), by breakthrough neutralization tests to detect other complete viruses (14, 18), and by CF tests for adenovirus-associated virus (AAV) types 1 to 4 (25). These incomplete satellite viruses were also sought by electron microscopic examination of Compton virus cultures directly after multiple passages in HEK and after fractionation of these passages in 30 to 50% CsCl gradients (2, 25, 28). Antiserum to the strain-purified virus was prepared in New Zealand white rabbits as previously described (18) and stored at −30 C.

Virus characterization. Commonly accepted procedures for virus characterization were carried out to place the isolate in the proper group (27, 31, 51). Nucleic acid type was determined by parallel titrations of the virus in HEK cells with regular maintenance medium and with medium containing 10^{-4} M 5-iodo-2′-deoxyuridine (final concentration); infectivity end points were read at 7 days (27). Nuclear and cytoplasmic viral nucleic acid was localized in four-chambered microculture slides (Lab-Tek Products, Naperville, Ill.) of HELF tissue inoculated with high dilutions of virus. The microcultures were incubated for 1 to 3 days under 5% CO_2/70% humidity at 37 C until minimal CPE was evident. Then they were washed 3× in phosphate-buffered saline (PBS), fixed in Carnoy's fixative for 30 s, hydrated through a series

of decreasing ethanol rinses, briefly immersed in 1% citric acid, rinsed in water, immersed in McIlvaine buffer at pH 4.95 for 5 min, and stained in 0.05% acridine orange in the pH 4.95 buffer (32). The slides were rinsed and mounted in this buffer and immediately examined for fluorescence at 350 nm under a 500× oil condenser and objective system.

The group CF antigen (hexon) was assayed by block titration against anti-AV 2 hexon mouse immune ascitic fluid (MIAF) in the standard CF test with overnight fixation of 5 units of complement (4, 8). Hemagglutinating antigens were measured by the standardized HA and HI tests with 0.01 M PBS diluent, pH 7.2, and 0.4% mammalian or 0.5% avian RBC suspensions (23, 24). HA tests were performed with a variety of mammalian and avian species at 37 C and 2 C to allow precise subgrouping of the virus (16).

Electron microscopy was carried out with infected HEp-2 cell cultures harvested at 3 to 4^+ CPE by three freeze-thaw cycles and clarified at 1,000 × *g* for 30 min. The virus was then concentrated 20-fold by pelleting at 105,000 × *g* for 1 h and prepared for examination by the pseudoreplica technique of Sharp (45). The grids were stained with 2.0% potassium phosphotungstate at pH 7.0 and examined at 60 kV in a Philips 201 electron microscope. Alternately, infected HEK cultures were harvested at 4^+ CPE, clarified, and prepared by pseudoreplica without prior concentration; these were examined at 80 kV in a Philips EM-300 microscope.

Chloroform stability was determined by mixing the virus with a final concentration of 5% $CHCl_3$ for 10 min at ambient temperature, separating the organic solvent layer by light centrifugation, and titrating the aqueous phase in HELF cells. Acid stability was determined by incubating the virus in citric acid buffer, pH 3.0, for 4 h at ambient temperature, neutralizing with phosphate buffer, pH 7.2, and titrating as above. Temperature stability was tested by incubating the virus in regular MEM at pH 7.0 at 50 C for 1 h and then titrating; for cationic stabilization, the virus was incubated with 1 M $MgCl_2$ (final concentration) in MEM at 50 C for 1 h and titrated. Control titrations of untreated virus were performed in HELF tissue simultaneously with the stability test titrations.

Microculture slides for viral inclusion stains were grown as for the acridine orange stain except that the cultures were allowed to progress to 3 to 4^+ CPE. They were then fixed in 10% neutral formalin and stained with either the May-Grunwald-Giemsa stain, or the Van Orden inclusion stain. The Van Orden inclusion stain required mordanting the cells in Zenker fluid followed by Lugol iodine and 5% thiosulfate solutions, staining with 0.5% Harris hematoxylin and 0.25% eosin Y by conventional hematoxylin and eosin stain methods, and counterstaining with a 0.5% ethanolic light green SF solution (Skip Van Orden, personal communication).

RESULTS

Virus isolations. The urine specimen collected on 30 March 1972 and the throat swab on April 29 1972 both yielded a virus in Laboratory 1, an AV from the former and cytomegalovirus (CMV) from the latter specimen. No other viruses were detected in the culture systems used. The CMV isolate was identified by its pathognomonic cytopathology. The adenovirus isolate was determined to be acid stable and to belong to Rosen's HA group 1 (42), but it was not neutralized by any of the group 1 antisera (types 3, 7, 11, 14, 16, 21).

In Laboratory 2, the AV was reisolated more than 2 years later. The CMV was not reisolated from the throat swab at this time because it did not survive the storage. Other testing yielded no additional isolates, despite repeated attempts to culture CMV from the urine specimen and AV from the throat specimen.

The reisolated AV was again found by initial testing to be a "typical" but "untypable" group 1A strain (16). Until proven otherwise, we assumed that the isolate could not be typed because it was a mixture of group 1 types. The isolate produced AV-like CPE in tissue cultures of human origin, hemagglutinins to 1:4096 HA titers with rhesus and vervet erythrocytes and no titers with other mammalian and avian cells, and hexon antigen to 1:32 CF titers when tested in a CF block titration with AV 2 antihexon MIAF. There was no reaction with antisera to herpes or respiratory syncytial virus in the CF test. The virus was only slightly inhibited (1:2 to 1:8) in both HI and SN tests by AV 11 and AV 21 antisera and was not inhibited in either HI and SN tests by the remaining group 1 antisera. Various combinations of group 1 sera, including an AV 11–21 serum pool, failed to neutralize the virus beyond a 1:8 serum dilution.

Virus characterization and strain analysis. The virus was purified as described in Materials and Methods; all characterization data were obtained both with purified virus and with early passage cultures. Sterility tests for bacterial, fungal, and mycoplasmal contaminants were negative at 28 days of incubation at 37 C. Serum neutralization breakthrough tests revealed only AV 34. CF tests for AAV 1 to 4 were negative, as were HA tests with human "O" cells at 4 C for AAV 4 (28).

The virus was inhibited by IUdR and produced yellow-green nuclear fluorescence with acridine orange stain, findings consistent with a double-stranded deoxyribonucleic acid virus (32, 51).

Supernatant fluids from virus-infected cells, harvested 2 days after 4^+ CPE, contained soluble CF and HA antigens characteristic of adenoviruses (16, 19–21, 31, 42, 51). In CF block

titrations, these fluids had 1:32 to 1:128 antigen titers against 1:512 to 1:1024 titers (optimal dilutions) of AV 2 hexon MIAF (8). In HA tests with a battery of avian and mammalian erythrocytes at 37 C and 2 C, only rhesus and vervet monkey red blood cells were agglutinated (Table 1). Complete hemagglutinins were detectable to 1:512 to 1:8192 titers with these erythrocytes, placing the virus in Rosen's HA group 1 (42) and, more specifically, in subgroup 1A (16).

As an additional test for AAV and to determine the morphological characteristics of the AV 34 soluble antigens, supernatant fluids from infected HEK cultures were fractionated on linear 30 to 50% CsCl gradients and the fractions were assayed for various biological activities. The HEK cultures were harvested by three cycles of freeze-thawing 2 days after 4^+ CPE, clarified by light centrifugation, and layered in 2-ml volumes onto 10-ml gradients. The gradients were centrifuged in a Beckman SW-36 rotor at 110,000 $\times g$ (average) for 23 h. Fractions collected by hole puncture from the bottom of the tube were assayed for refractive index by a Bausch & Lomb Abbé refractometer, for AAV and AV morphological structures by electron microscopy (after removing the salt by dialysis), for infectivity titers in HEK, for hexon antigen by CF tests with AV 2 anti-hexon MIAF, and for complete and incomplete hemagglutinins by HA tests in PBS and in PBS with 1% rhesus RBC-absorbed AV 16 equine antiserum. The results are shown in Fig. 1. No AAV-like structures were found in the fractions with densities of 1.39 and 1.43 g/ml, the density in cesium of AAV 1 to 3 and AAV 4, respectively (25, 28). The adenovirion, with typical morphology and CF and HA activities, had a buoyant density of 1.340 g/ml. Three soluble components were found: hexon, with group-specific CF activity, had a density of 1.304 g/ml, dodecon, a complete hemagglutinin, 1.295 g/ml, and another complete hemagglutinin tentatively identified by electron microscopy as a dimer of fiber components, 1.206 g/ml. No incomplete HA components detectable in type 16 heterotypic serum diluent were found.

Negative contrast electron microscopy on clarified virus culture fluids revealed AV-like particles with cubic icosahedral symmetry and an average diameter, excluding projections, of 73 nm (26). Hexon and vertex capsomeres and a few fiber projections were clearly discernible on the virions. No envelope or limiting membrane was present.

The virus was chloroform and acid stable and, according to the methods used, was partially heat-labile (0.5-log drop) at pH 7.0 with or without 1 M $MgCl_2$ (51). A detailed temperature stability study, with virions not protected by the protein and buffering components of the culture medium, was not carried out.

TABLE 1. *Hemagglutination properties of the Compton strain*

Erythrocyte species	HA titers[a] at:	
	37 C	2 C
Vervet	512-8192	64-2048
Rhesus	512-4096	128-2048
Human "O"	<1-1	<1
Rat	<1-4	1
Rat-HS[b]	<1-4	<1
Mouse	<1-1	<1
Gerbil	<1-1	1
Guinea pig	<1	<1
Dog	<1	<1
Chicken	<1	<1
Goose	<1	<1
Turkey	<1	<1
Cow	<1	<1
Sheep	<1	<1

[a] An HA titer is defined as the dilution factor of the highest dilution of antigen causing complete hemagglutination; range of titers listed includes data on three passages, each tested with erythrocytes from three animals (16).

[b] Rat erythrocytes with virus diluted in heterotypic serum (HS) diluent (PBS containing 1% rat RBC absorbed AV 6 equine antiserum) (16, 42).

The virus readily replicated to 10^4 to 10^7 mean tissue culture infective doses per 0.1 ml at 14 days in tissue cultures of human origin only: WI-38, HELF, RU-1, KB, HEp-2, HeLa, and HEK. In all of these the cytopathology was typically AV-like (27, 31), although the CPE in fibroblast cultures began as a nondescript granular degeneration and only became AV-like in subsequent passages. In microcultures of HELF tissue, the virus produced Cowdry type B basophilic intranuclear inclusions (Fig. 2) concomitant with fully developed cytopathology (27, 39). The virus did not replicate in cultures of simian origin in the absence of adventitious simian viruses, but did replicate in MK cultures with AV-like CPE in the presence of simian virus-40. The virus thus was a complete virus in terms of replication (in human tissue) but required "potentiation" for growth in its unnatural host system (MK) (40, 41). The virus did not produce lesions on the chorioallantoic membrane of 12-day embryonated eggs, replicate in the amniotic or allantoic fluids of 11-day eggs

FIG. 1. *Equilibrium cesium chloride gradient showing the absence of AAV and the buoyant densities of the AV 34 virion and soluble CF and HA components. Complete hemagglutinins were measured in PBS diluent, and incomplete hemagglutinins were titrated in type 16 heterotypic serum (HS 16) diluent.*

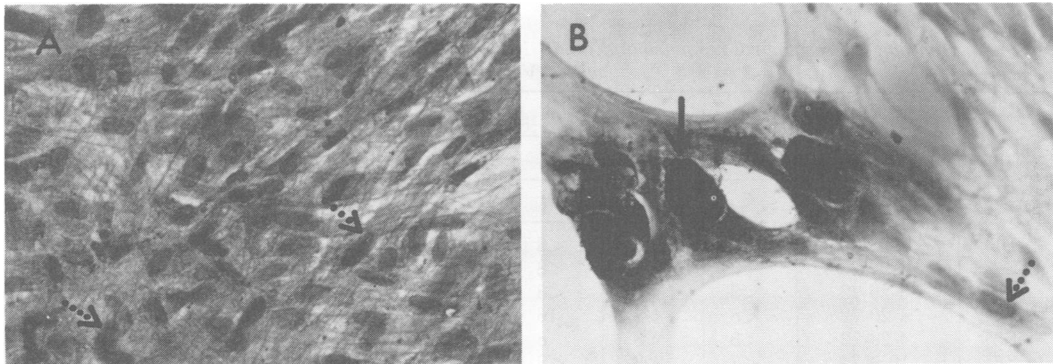

FIG. 2. *HELF tissue culture slide with compartment of normal cells (A) and 3 compartments of cells infected with AV 34 for 6 days (B). Slide was stained with the Van Orden inclusion stain to contrast normal nuclei (dotted arrows) with the type B basophilic intranuclear inclusions (solid arrows). Final magnification, ×250.*

even after repeated passage, or appear to be pathogenic for 1-day-old mice inoculated intracerebrally and intraperitoneally (27, 31, 51).

Antigenic characterization of AV 34 was carried out by HI and SN tests with the virus and reference equine antisera to prototype strains of AV 1 to 33 (20), and, reciprocally, by HI and SN tests with AV 34 rabbit antiserum and AV 1 to 33 antigens. AV 34 was not inhibited in HI tests or neutralized in SN tests by AV 1 to 33 antisera; conversely, AV 34 antiserum failed to inhibit or neutralize AV 1 to 33 antigens although it had homologous titers of 1:320 (HI) and 1:640 (SN) (Table 2). In addition, AV 34 did not produce precipitin lines in counterelectrophoresis tests with AV 3, 7a, and 11 antipenton and anti-dodecon rabbit sera (17).

Serologic study of patient. Five serum specimens from the patient were available from before and after the collection date of the urine specimen which yielded AV 34. An additional serum was obtained on 9 October 1974. The HI and SN titers of these sera are shown in Table 3. Titers obtained with early passage cultures were identical to those obtained with strain-purified passages. Serum antibody titers to AV 34 were not high, even at the peak, but they clearly indicate infection in the patient. At the same time, HI tests with the other group 1 serotypes indicate that these viruses, some of which are very common infectious agents in children and young adults, were not currently infecting the patient.

The group 1 adenovirus HI titers in Table 3 also indicate that heterotypic antibody responses may occur with AV 34 infection. Low levels of HI antibody were detected with AV 11, 16, and 21 at the time of the peak homologous AV 34 titers. Interestingly, two of these were the same serotypes for which the reciprocal test (AV 34 virus against reference equine antisera to AV 11 and 21) had earlier shown low-level cross-reactions. Infection with AV 11, 16, or 21 probably did not account for the heterotypic HI titers because SN tests with these viruses were negative (<1:8).

The patient's multiple sera were additionally tested by CF against a battery of respiratory viruses and by HI against coronavirus OC-43 (Table 4). This was done to document both the CMV infection and the presence or absence of other common virus infections. CMV, isolated from a pharyngeal swab collected on 29 April 1972 initiated a CF antibody response from April 20 onward. The time of the peak antibody titer cannot be determined but was apparently after April 29 because the titer (1:512) 2.5 years later was a dilution higher than that of April 29 (1:256). In our tests, CF titers to CMV infection often peak at 1:512 or higher.

During the same time period as the CMV infection, the patient was apparently infected with respiratory syncytial virus (RSV). The RSV infection, as shown by a very typical CF antibody response to RSV, may have elicited

TABLE 2. *Relationship of Compton strain to AV types 1-33*

AV type	Antiserum			
	AV types 1-33 (equine)		AV 34 (Compton) (rabbit)	
	HI	SN	HI	SN
1-33	–[a]	–[a]	<10	<10
34 (Compton)	<10	<10	320	640

[a] See reference 20 for homologous and heterologous titers.

TABLE 3. *Homologous serological response and group 1 AV heterotypic responses of patient to infection with AV 34*

Serum	Date	Serum antibody titer[a]							
		HI test							SN test
		3	7a	11	14	16	21	34	34
S1	2-23-72	<8	<8	<8	<8	<8	<8	<4	<4
S2	3-9-72	<8	<8	<8	<8	<8	<8	16	8
S3	3-20-72	<8	<8	<8	<8	<8	<8	32	16
S4	4-20-72	<8	<8	16	<8	8	8	128	64
S5	4-29-72	<8	<8	8	<8	<8	<8	32	32
S6	10-9-74	<8	<8	<8	<8	<8	<8	8	4

[a] HI antibody titer is listed as the dilution factor of the highest dilution of serum completely inhibiting 4 HA units of virus per 0.025 ml in 1 h at 37 C. SN antibody titer is listed as the dilution factor of the highest dilution of serum causing a 2^+ reduction in CPE from that observed in the working dilution (virus control) in 3 days.

TABLE 4. *Serum antibody titers of patient to common respiratory viruses*

Serum	Date	Serum antibody titer														
		CF test														HI test
		Influenza		Parainfluenza				Mumps	Rubeola	RSV	Herpes 1	*M. pneumoniae*	AV[b]	CMV	Coronavirus 229E	Coronavirus OC-43
		A	B	1	2	3	4									
S1	2-23-72	0[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2	3-9-72	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0
S3	3-20-72	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0
S4	4-20-72	8	0	0	0	0	0	0	0	16	0	0	64	64	0	0
S5	4-29-72	8	[illegible]	8	16	8	0	0	0	64	0	0	32	256	0	0
S6	10-9-74	8	32	0	0	0	0	0	0	8	0	0	0	512	0	64

[a] 0, <8.

[b] Purified AV 2 hexon antigen (8), measuring in this test strictly the adenovirus-group specificity.

the low-level heterotypic or anamnestic CF titers to influenza A and parainfluenza 1, 2, and 3 seen in the April 29 serum. We have previously observed such titer patterns in complex illnesses, particularly in young adults with altered immunological systems.

The CF antibody titer to influenza B and the HI titer to coronavirus OC-43 reveal separate infections with these agents in the interval between May 1972 and October 1974. Both infections were probably in 1974 because the significant levels of antibody suggest recent infection and because both viruses were documented to be endemic in the patient's area at that time. These infections therefore did not impinge on the AV 34 infection which started in late February or early March 1972 (Table 3, HI and SN, and Table 4, CF) or on the CMV and RSV infections which began in late March or in April 1972.

DISCUSSION

The etiological relationship between AV 34 infection and the patient's febrile illness is unclear. Multiple viruses were shown to be infecting the patient during March and April 1972 and in June there was clinical and pathological evidence of a rejection reaction in the transplanted kidney (see Materials and Methods). The temporal relationships between mild liver dysfunction, fever, and serum antibody responses to AV 34 and CMV infection are shown in Fig. 3. Bilirubin levels varied between 0.3 and 1.1 mg/100 ml and were never elevated. The April 13 peak in temperature, SGOT, and LDH and the April 24 peak in alkaline phosphatase occurred a month or more after the first serological evidence of AV 34 infection. The patient was experiencing at least two other causes of fever (RSV and CMV infections) and at least four other potential causes of liver function abnormality. The latter include right-sided congestive heart failure with congestion of the liver, treatment with azathiaprine (75 to 150 mg per day) and isoniazid (300 mg per day), and the CMV infection (1).

It is possible that the AV 34 infection was responsible for at least some of the early part of the 3-month-long fever. The detection of HI and SN antibody in early March indicates infection at that time; the isolation of the virus from urine on March 30th when the antibody titer was still rising shows infection then. Since multiple urine specimens were not obtained it is impossible to determine the total duration of virus shedding.

The origin of the AV 34 remains a fascinating unknown. No other untyped AVs in our 15-year collection are inhibited or neutralized by type 34 antiserum. Temporal data suggests that, in the case at hand, the virus may have been latent in the transplanted kidney: CF, HI, and SN AV antibodies were not present in the recipient at the time of transplant, but were detected 16 days later. Unfortunately, serum and other specimens from the donor were not available to help determine if he had had prior infection with the virus. If the virus had been latent in the transplanted kidney, it could have become active in the new host as a consequence either of his immunosuppression therapy and/or of impaired immunity resulting from chronic renal failure. The findings in this case are akin to those of another renal transplant case from whom a new AV type was isolated from the lung and kidney (36). Also in that case, which terminated in a fatal pneumonitis, a latent virus was suspected to have been present in the donor kidney.

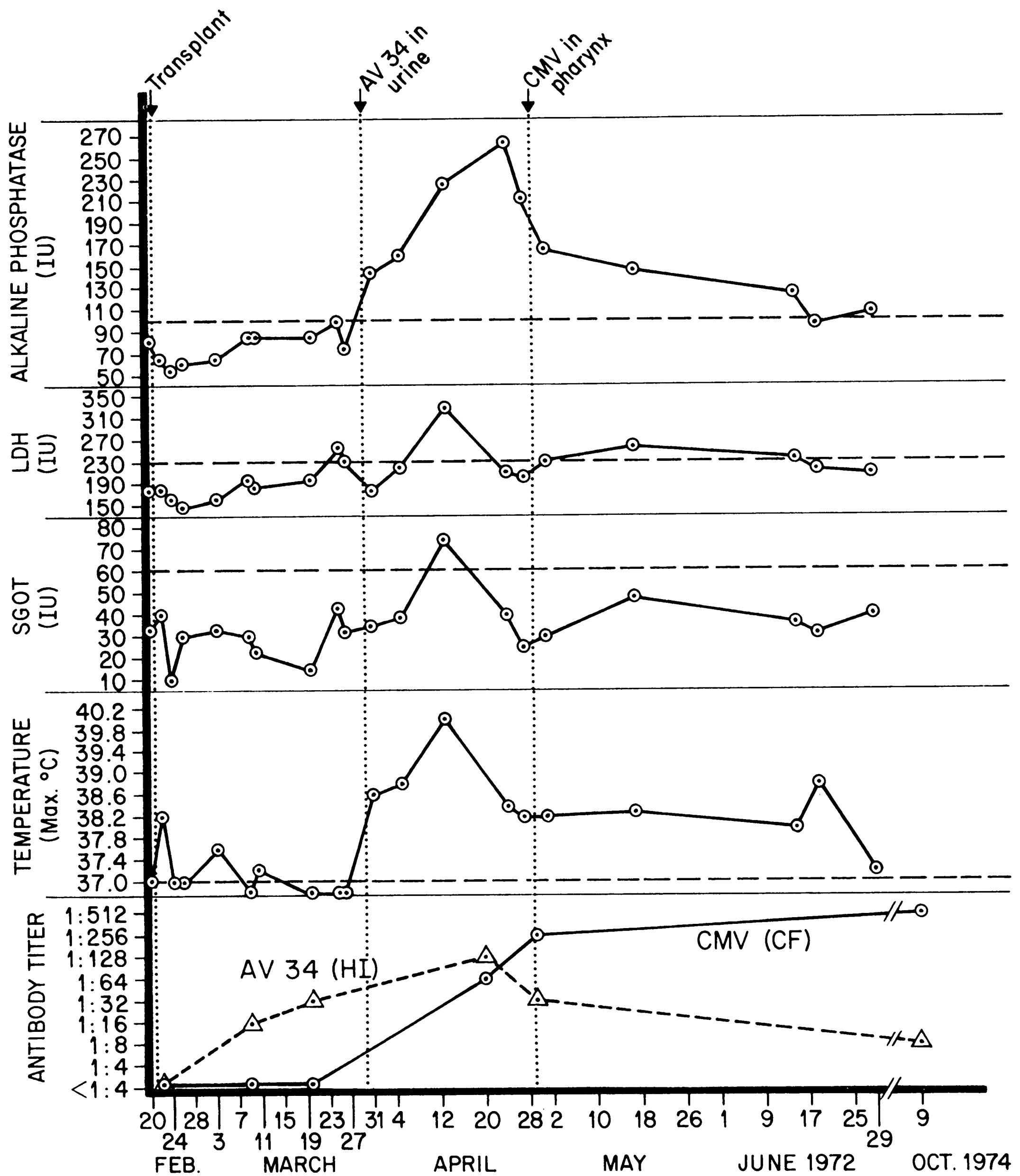

FIG. 3. *Temporal relationship of certain serum enzyme concentrations (expressed in International Units) with fever, HI antibody titers to AV 34, and CF antibody titers to CMV. The dashed line in each segment indicates the upper limit of the normal range for alkaline phosphatase, lactic acid dehydrogenase (LDH), and serum-glutamic-oxalacetic acid transaminase (SGOT) enzymes or the normal temperature. The vertical dotted lines indicate the dates of the renal transplant and of the collection of urine which yielded AV 34 and of throat swab which yielded CMV.*

The isolation of AVs from urine or kidney tissue and their association with urinary-tract disease has until recently been largely ignored (49). AV type 1 has been isolated from the urine of an infant with URI (11). Types 1, 2, and 7 have been isolated from kidneys of infants at autopsy (33). Types 3 and 4 were associated with hematuria during pharyngoconjunctival fever in children and young adults (50). Type 5 was isolated from the kidney of an infant after a fatal generalized illness (7). Types 4 and 7 have been isolated from the urine of naval recruits

with rubelliform illness (12) and AV 7 from urine of a child with acute leukemia (15). In addition, type 7 has been isolated from the kidney of an infant with a fatal generalized disease (3) and has been associated with acute nephritis during fatal and severe pneumonia in infants and children (5) and young adults (46). Type 11 is clearly an etiological agent of acute hemorrhagic cystitis in children (29, 34, 35, 37, 38); this interesting but heretofore rare serotype has recently been reviewed (22) and its unusual hemorrhagenic characteristics discussed (48). Type 21 also has isolated from the urine of children with acute hemorrhagic cystitis (35). In our laboratory, we have isolated AV 1, 2, 3, 5, 7, 11, and 31 from the urine of infants and children with cystitis (type 11 only) or generalized illnesses (unpublished data). But of all these types, the group 1 AVs, notably types 3, 7, 11, and 21, appear to have a proclivity for urinary-tract infection.

Thus, it is evident that many adenovirus types can infect the kidney and possibly other parts of the urinary tract. In the present case, the isolation of AV 34 from the urine does not implicate any particular site of infection. Review of the biopsy specimen of the transplanted kidney revealed no AV-like inclusions; however, the small size of the specimen did not allow an optimal examination, and also the biopsy was obtained 3 months after the onset of the AV 34 infection. In the case of another new group 1 AV, however, the virus was not only isolated from the kidney but could be seen directly by electron microscopy and by inclusion-body morphology in these kidney sections (36). Another group 1 AV, type 11, was recently isolated from the urine of three patients with hemorrhagic cystitis following renal transplantation (30).

Proof that the AV 34 (Compton) strain was an adenovirus fits the accepted definitions of the AV group (9, 10, 43, 44) and the current classification schemes for grouping viruses (26, 31, 51). The virus was a 73-nm icosahedral particle with double-stranded DNA and a buoyant density in CsCl of 1.34 g/ml; it was chloroform and acid stable and partially heat labile; it replicated only in human tissue cultures, producing soluble CF and HA antigens typical of adenoviruses.

We conclude that the Compton strain is a new adenovirus serotype, AV 34, which has no serological relationship by HI and SN tests to other recognized human serotypes.

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