Characterization by Hemagglutination-Inhibition of Adenovirus 11 Strains from Urine of Patients with Hemorrhagic Cystitis[1] (37424)

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Recently, adenovirus type 11 has been implicated as one of a number of etiologic agents producing acute hemorrhagic cystitis illnesses in children (1, 2). The recovery rate of adenovirus type 11 from the urine of children with this disease was approximately 15% (Mufson *et al.*, unpublished). This is of particular interest since adenovirus type 11 has been rarely isolated from the oropharynx or stool of children with acute respiratory tract diseases or from the conjunctivae in acute conjunctivitis (3–5). Not only the association of adenovirus type 11 infection with acute hemorrhagic cystitis but also the high recovery rate raised the question whether these isolates differ antigenically from the prototype strain (6). This report describes the antigenic relationships defined by hemagglutination-inhibition of four adenovirus type 11 strains recovered from urine and the prototype adenovirus type 11 strain.

Methods. Virus strains. Adenovirus type 11 strains were isolated in HEp-2 cells from urine of patients with acute hemorrhagic cystitis hospitalized at the Cook County Hospital. These strains, obtained during three different years, were designated as the Scott strain (1970), the Dean and Briggs strains (1971), and the Heard strain (1972). The prototype Slobitski strain, which was used for comparative studies, was originally isolated from stool. It was obtained from the Reference Reagents Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland (6).

Infectivity titrations. Fifty per cent end point infectivity titrations of each virus strain were determined in human embryonic kidney (HEK) and HEp-2 cells. The cell lines were maintained as previously described (7). Tenfold dilutions of virus were made in serum-free tissue culture media and 0.2 ml of each dilution inoculated into each of two roller tube cultures. The cultures were examined twice weekly for five weeks for cytopathic effects and the media was changed at these times. End points were calculated according to the method of Reed and Muench (8).

Antigen preparation. Hemagglutination (HA) antigen for each strain was produced in HEp-2 cells as previously described (2). Urine strains were passaged in HEp-2 cells three times to increase HA antigen titers. The Slobitski strain was passaged three times in HEK cells and then twice in HEp-2 cells. Virus harvests were frozen and thawed and stored in aliquots at −70° until used.

Antisera. Specific antiserum for each adenovirus type 11 isolate was produced in rabbits by initial intravenous administration of 2 ml of virus which usually titered 10^6 TCD_{50}/0.2 ml followed by two 1-ml intravenous doses of virus given at weekly intervals. Rabbits were bled 4–10 weeks subsequent to the start of immunization. Each antiserum was produced in a single rabbit. Antiserum for the Slobitski strain produced in rabbits was obtained from the Reference Reagents Branch. Prior to use, all sera were inactivated at 56° for 30 min, diluted 1/10 with phosphate-buffered saline (pH 7.2), and absorbed with a 50% suspension of rhesus monkey erythrocytes at

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4° for 1 hr to remove nonspecific agglutinins (9).

Hemagglutination-inhibition test. Cross hemagglutination-inhibition (HI) tests were carried out using standard microtiter procedures (9). "V" type microtiter plastic disposable plates were employed. One per cent rhesus monkey erythrocytes and four HA units of antigen were used. Virus and antiserum were incubated together for various times to detect differences in rates of reaction. The incubation times used were 4 min, 30 min, 5 hr and 21 hr. All HI tests were run in duplicate.

Calculations. Antigenic relatedness was estimated using the formula:

$$\text{Per Cent Relative Similarity of Virus Strains} = (\gamma_1 \times \gamma_2)^{1/2} \times 100,$$

$$\text{where } \gamma_1 = \frac{\text{Reciprocal heterologous titer of virus 2}}{\text{Reciprocal homologous titer of virus 1}},$$

$$\gamma_2 = \frac{\text{Reciprocal heterologous titer of virus 1}}{\text{Reciprocal homologous titer of virus 2}}.$$

A value of 35% or less was interpreted as distant relatedness.

Results. Infectivity titers. Infectivity of adenovirus type 11 strains assayed 10 to 100-fold higher in HEK than HEp-2 cells (Table I). Two strains, Dean and Slobitski (prototype strain), showed similar titers in the two cell lines, but the other three strains assayed 100-fold higher than the prototype strain in HEK cells. Infectivity:hemagglutination ratios for the urine strains varied between 10^3 and 10^5.

Relation of reaction time to HI antibody titer. HI titers of antibody to each strain were lowest at the shortest reaction time and increased between four and sixteenfold after 5 hr incubation time (Table II). Little increase in titer developed after 5 hr reaction. HA antigens did not decay during an incubation period of 21 hr.

Strain comparison. In general, minimal strain differences were found among the five adenovirus type 11 isolates tested. Some strain variation was evident at the 4-min incubation time (Table III). The Scott strain showed 35.7% relatedness to the Heard and Slobitski strains, and 50% relatedness to the Briggs strain. At the 30-min incubation time, the titer differences between the Scott and Slobitski strains were less pronounced. The Briggs strain differed slightly in relatedness to the Heard and Slobitski strains at the 4-min incubation time. The slight differences between the prototype Slobitski strain and the strains from urine which were manifest at the 4-min incubation time tended to disappear when longer reaction periods were used. After 21 hr incubation time, no differences between strains were detectable.

Discussion. These data suggest that minimal antigenic differences exist between the Slobitski prototype strain of adenovirus type 11 and randomly selected strains of adenovirus type 11 recovered from the urine of children with acute hemorrhagic cystitis. Similarly only slight differences between the four urine strains were detectable. Although no differences were found between the two urinary adenovirus 11 strains Dean and Briggs, both isolated in 1971, differences in antigenic relatedness were found between the

TABLE I. Comparison of Infectivity and Hemagglutination Titers of Adenovirus Type 11 Strains Isolated from Urine.

Strain	Infectivity titer $\log_{10}$ TCD_{50}/0.2 ml in		Hemagglutination titer reciprocal	Infectivity[a] hemagglutination ratio
	HEK	HEp-2		
Dean	6.0	5.5	32	10^5
Scott	5.5	3.5	8	10^4
Briggs	6.5	4.5	128	10^4
Heard	5.5	3.5	128	10^3
Prototype (Slobitski)	6.5	5.5	64	10^4

[a] Infectivity assayed in HEK.

TABLE II. Cross HI Titers of Five Adenovirus Type 11 Strains Reacted at Various Times.

	Reciprocal HI titers using indicated specific antisera at specified reaction times																			
	4 min					30 min					5 hr					21 hr				
Antigen	Dean	Scott	Briggs	Heard	Slobitski	Dean	Scott	Briggs	Heard	Slobitski	Dean	Scott	Briggs	Heard	Slobitski	Dean	Scott	Briggs	Heard	Slobitski
Dean	40	80	20	80	80	80	80	40	160	80	160	160	80	320	320	160	320	80	320	320
Scott	20	80	<20	20	<20	40	160	20	40	40	160	320	40	160	160	160	320	80	160	160
Briggs	40	40	<20	80	40	80	160	20	160	80	320	320	80	640	320	320	320	80	≦640	≦640
Heard	40	40	20	40	20	80	160	20	160	80	160	320	80	640	320	320	320	80	≦640	≦640
Slobitski	40	40	<20	40	80	80	80	<20	80	160	320	320	40	320	160	ND[a]	ND	ND	ND	ND

[a] ND, not done.

TABLE III. Relatedness of Five Adenovirus Type 11 Strains.

	Comparison of relatedness using indicated antisera at specified reaction times[a]																			
	4 min					30 min					5 hr					21 hr				
Antigen	Dean	Scott	Briggs	Heard	Slobitski	Dean	Scott	Briggs	Heard	Slobitski	Dean	Scott	Briggs	Heard	Slobitski	Dean	Scott	Briggs	Heard	Slobitski
Dean	100					100					100					100				
Scott	70.7	100				50	100				50	100				100	100			
Briggs	100	50	100			141	100	100			141	70.7	100			141	100	100		
Heard	100	35.7	70.7	100		100	50	100	100		70.7	50	100	100		141	50	100	100	
Slobitski	100	35.7	70.7	70.7	100	70.7	35.7	50	50	100	200	100	50	100	100	ND[b]	ND	ND	ND	ND

[a] Expressed as per cent; formula in text.
[b] ND, not done.

Scott strain of 1970 and the Heard strain of 1972. These findings suggest that the adenovirus 11 strains recovered from urine may have shifted slightly in antigenic structure during a several-year period.

In determining differences between virus strains, the 4-min incubation time provided the most sensitive measure, as has been previously reported for rubella virus strains (10). Casals reported that homologous reactions predominate at short reaction times, but at longer reaction times cross reactions develop (11). In our studies at the 4-min reaction time, both the Scott and Slobitski strains showed the widest differences in relatedness from the other strains tested.

That antigenic variation among adenovirus type 11 strains recovered from urine or stool exists and could be shown by cross hemagglutination-inhibition tests suggests that these isolates may have other differing properties. Whether the urinary isolates possess a special affinity to infect cells of the urinary bladder remains to be investigated.

Summary. To determine whether adenovirus type 11 strains recovered from urine of children with acute hemorrhagic cystitis differed antigenically from the prototype strain, these strains were examined by cross HI tests. Four adenovirus type 11 strains (Dean, Scott, Heard, and Briggs) isolated from urine and the prototype Slobitski strain were tested. Reaction times of virus with antibody varied between 4 min and 21 hr. At the shortest reaction time, strain differences were most pronounced. The Scott and Briggs urine strains showed less resemblance to the Slobitski strain and other urine strains. At longer reaction times, titer differences disappeared. Except for these differences, which were not marked, the adenovirus type 11 strains from urine appeared antigenically similar to the prototype strain.

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