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Remarkably Homogeneous Population of Adenovirus Type 3 and 7 Genome Types

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A total of 270 frozen adenovirus isolates, obtained from January 1981 to December 1986, were grown in KB cells. DNA was successfully prepared from 248 of these isolates, and the prepared DNA was digested initially with *Sma*I restriction endonuclease and then analyzed by agarose gel electrophoresis. Restriction fragment patterns showed that 68 of these DNAs were either adenovirus type 3 (Ad3) or Ad7. Further analysis by digestion with *Bam*HI showed that 24 isolates were Ad3a and 44 isolates were Ad7b. When Ad3 isolates were digested with *Sma*I or *Pst*I, the restriction fragment patterns of the 24 isolates were identical. Of these 24 isolates, 20 (83%) also gave identical patterns when digested with *Hin*dIII, and the patterns of the remaining 4 isolates were different from that of the 20 but identical to each other. All Ad3 isolates were obtained in 1982, 1983, and 1986. All the 44 Ad7b isolates gave identical fragment patterns when digested with *Bgl*I or *Pvu*II, and 43 of 44 (98%) isolates gave identical patterns when digested with *Sst*II. We obtained at least two Ad7 isolates during each of the 6 years studied. Although considerable genome heterogeneity within adenovirus serotypes and subtypes has been reported, our population of isolates is remarkably homogeneous. This homogeneous set of genome types was obtained from diverse anatomical sites and was associated with a broad spectrum of clinical syndromes.

There has been considerable recent worldwide interest in the use of restriction endonucleases to investigate the genomic variability of adenoviruses. A single base change in the viral DNA can potentially be observed as an altered restriction enzyme fragment pattern in an agarose gel, and thus, various patterns with different enzymes have been used to define specific genomic variants or viral subtypes. This ability to describe subtypes is interesting and useful for epidemiological purposes in determining the presence and relationship of specific variants circulating in a community and possibly in evaluating the relative virulence of different subtypes.

In this study, we have grown virus and prepared viral DNA from 248 adenovirus isolates obtained in the Diagnostic Virology Laboratory at Cardinal Glennon Children's Hospital in St. Louis, Mo., between 1981 and 1986. Of these isolates, 204 were typed by comparison of their restriction enzyme fragment patterns with standard patterns. Within this group, 24 isolates were typed as adenovirus type 3 (Ad3) and 44 isolates were Ad7. These isolates were further digested with additional restriction enzymes for purposes of subtyping. In contrast to previous reports, our subtyped Ad3 and Ad7 populations were found to be extremely homogeneous.

KB cells (approximately 1×10^6) were plated in 6-cm-diameter tissue culture dishes and incubated at 37°C in 5% CO_2 until confluent (24 to 48 h). The growth medium was removed, and cells were infected with 150 to 250 μl of a viral isolate and further incubated at 37°C with occasional tilting for 1 h. Cells were fed with 5 ml of medium (Dulbecco modified Eagle medium with nonessential amino acids, 10% fetal bovine serum, and 50 mg of gentamicin per ml) and incubated as described above until 50 to 100% of the cells were showing cytopathic effect (48 to 72 h).

DNA was prepared from infected cells essentially by the method of Kemp et al. (6). Cells were harvested, washed with phosphate-buffered saline, suspended in 100 μl of phosphate-buffered saline, and then lysed by the addition of 0.5 ml of lysis buffer (1% sodium dodecyl sulfate, 1% 2-mercaptoethanol, 10 mM Tris hydrochloride [pH 8.0], 150 mM NaCl, 5 mM EDTA) and incubated at 37°C for 15 min. Proteinase K (200 μg) was added, and incubation was continued for 60 min. This digestion mixture was then extracted with an equal volume of phenol-chloroform-isoamyl alcohol (24:24:1), and the large clump of cell DNA was removed and discarded following centrifugation to separate the phases. The DNA in the aqueous phase was precipitated with 2 volumes of ethanol at −20°C for 30 min. The precipitate was recovered by centrifugation, washed with ethanol, and then dried in vacuo. DNA was dissolved in 0.5 ml of TES buffer (10 mM Tris hydrochloride [pH 8], 1 mM EDTA, 150 mM NaCl), digested with RNase A (25 μg) and RNase T_1 (25 U) at 37°C for 60 min, extracted twice with phenol-chloroform-isoamyl alcohol as described above, extracted with ether, and precipitated with ethanol. The recovered DNA was dissolved in 200 μl of H_2O.

Purified DNA (1 to 2 μg) was digested with restriction enzymes according to manufacturer specifications with 10 to 20 U of enzyme. Digested DNA was subjected to electrophoresis in 1.2% agarose gels containing ethidium bromide for 17 h at 30 V. Gels were photographed under UV light.

Some isolates were serotyped by neutralization, and restriction patterns from these isolates were used for comparison with unknown patterns from isolates and with published patterns.

A total of 270 isolates collected from January 1981 to December 1986 were inoculated into KB cells as described above. Viral DNA was successfully purified from 248 (92%) of these isolates. The yearly breakdown of isolates and the number that grew in KB cells and the genome types of these

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TABLE 1. Summary of adenovirus genome types

Genome type	No. of isolates					
	1981	1982	1983	1984	1985	1986
1/2	10	11	7	10	18	14
3a		13	3			8
4	1				1	
4a	2					
5	5	2	5	1	6	
6	2	1				1
7b	4	13	2	2	8	15
11		1	1			
31	4	3	3		5	2
34/35/11	2		1		1	3
37	3				5	2
41						2
Group						
A					1	
B		2	1			
C	4	2	10	8	3	4
B/C	3					
Other		2	4		1	
Total	42	52	43	29	52	52
Total that grew in KB cells	40	50	37	21	49	51

isolates as determined by comparison with standards or with published patterns are shown in Table 1. By far the most common types were types 1 and 2, as would be expected from a pediatric population (3). The genomes of these two types cannot be distinguished when digested with *Sma*I restriction enzyme (4). The next most common types were Ad7, which accounted for 17.7% of the 248 typed isolates, and Ad3, which accounted for 9.8% of the typed isolates. There were 45 isolates (10% of the total) which could not be easily typed after digestion with *Sma*I, but most of these isolates could be assigned to a serotype group on the basis of their fragment pattern (Table 1). Group C adenoviruses are known to show considerable genome variation (1, 4, 5), and the subtypes can be identified only after the digestion of DNA with several restriction enzymes. Seven isolates had complicated or uncharacteristic patterns that did not seem to fit a particular group pattern and were thus classified under other genome type in Table 1.

Figure 1 shows the yearly distribution of Ad3 and Ad7 isolates from 1981 through 1986. As has been shown previously (8, 9), the isolation of Ad3 and Ad7 serotypes fluctuates considerably from year to year. Although these two serotypes generally fluctuate in concert with each other, Ad7 is usually present every year whereas Ad3 often disappears for a year or more and then reappears in the same population, as was the case with the isolates in this study.

Table 2 shows the results of digestion of all 24 Ad3 isolates from the 23 patients represented in Fig. 1. DNA was initially digested with *Bam*HI to subtype the isolate. All of our isolates were Ad3a as determined by comparison with the published fragment profiles of Wadell (7). Digestion of all 24 DNA samples with *Sma*I revealed a fragment pattern identical to *Sma*I cleavage pattern III of Bailey and Richmond (2). Digestion of all Ad3a DNAs with *Pst*I yielded a cleavage pattern not previously described and which we have designated pattern E. Finally, digestion of these DNAs with *Hin*dIII gave two distinct patterns, neither of which has been previously described. DNA from four of the Ad3a isolates yielded the *Hin*dIII pattern shown for isolate 82-1421 in Fig. 2 and which is designated pattern U in Table 2. The remaining 20 *Hin*dIII DNA fragment patterns were identical (represented by the patterns for isolates 82-3053 and 86-3996 in Fig. 2), and the pattern is designated pattern V in Table 2. Thus, 4 of the 24 Ad3a isolates were indistinguishable from each other by this analysis. The remaining 20 isolates were distinguishable from the first group by their *Hin*dIII patterns but were identical to each other.

Table 3 shows the results of restriction enzyme digestion of the Ad7 isolates. The *Bam*HI pattern was characteristic of Ad7b as defined by Wadell (7). All 44 isolates gave identical fragment patterns when digested with *Bgl*I or *Pvu*II restriction enzymes. The *Bgl*I and *Pvu*II patterns were patterns B and Y, respectively, as defined by Bailey and Richmond (2). DNA digestion with *Sst*II revealed 43 isolates with pattern III (2) and 1 isolate with pattern IV. Figure 3 shows representative Ad7b restriction patterns, all of which were identical for each enzyme used in this analysis except for *Sst*II. Isolate 82-1218 gave a unique pattern with *Sst*II which distinguished it from the other 43 isolates. Table 4 summarizes the data presented in Tables 2 and 3 and illustrates the homogeneous nature of our Ad3 and Ad7 isolates. Of our Ad3 isolates, 83% belonged to the newly described $Ad3a_7$ subtype, and 98% of our Ad7 isolates belonged to the previously described $Ad7b_2$ subtype.

In contrast to previous reports of genetic heterogeneity of certain populations of adenovirus isolates, particularly Ad3 and Ad7, our present study shows that the Ad3 and Ad7 isolates obtained at the Diagnostic Virology Laboratory at Cardinal Glennon Children's Hospital constitute a remarkably homogeneous population.

A previous report (2) defined nine Ad3 genome subtypes in a population of 29 isolates from Manchester, England. By using the same restriction enzymes for analysis, we found only two genome subtypes of Ad3 for our 24 isolates collected from 1981 to 1986 in St. Louis, Mo. Of these isolates, 83% belonged to a single subtype, now designated $Ad3a_7$. The remaining isolates also gave restriction patterns that have not previously been described and have been classified as belonging to a subtype designated $Ad3a_6$.

For a population of 44 Ad7 isolates, a previous report (2) defined three circulating subtypes which were about equally represented among the isolates. In the present study, we found only two genome variants among our 44 Ad7 isolates, and 43 (98%) of these belonged to a single subtype ($Ad7b_2$).

The global distribution of adenovirus types and subtypes is becoming well characterized through the efforts of molec-

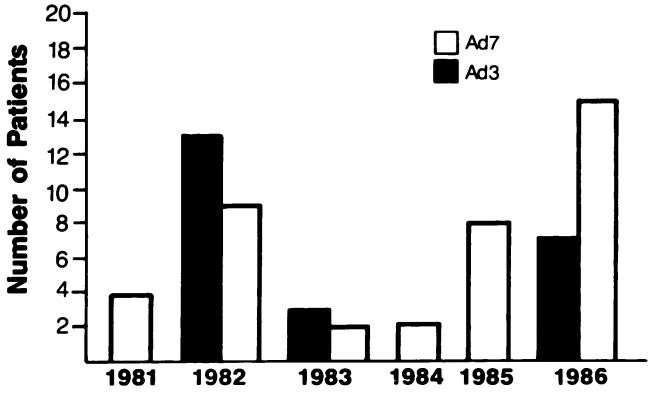

FIG. 1. Number of patients with Ad3 or Ad7 from 1981 to 1986 in St. Louis, Mo.

TABLE 2. Ad3a isolates from 1981 to 1986

Isolate no.	Specimen	Diagnosis	*Hin*dIII restriction pattern[a]
82-963	Throat	Fever of unknown origin	U
82-1420	NP[b]		U
82-1421	NP		U
82-1694	Eye swab	Cellulitis	V
82-1948	Conjunctiva swab	Conjunctivitis	U
82-2941	Throat	Pharyngitis	V
82-3033	NP	Fever of unknown origin	V
82-3053	Throat	Upper respiratory infection	V
82-3370	Sputum	Pneumonia	V
82-3412	Throat		V
82-3486	Throat	Fever of unknown origin	V
82-3503	Urine[c]	Rule out cytomegalovirus	V
82-3586	Throat		V
83-1941	NP		V
83-4029	NP	Neutropenia	V
83-4260	Stool	Respiratory distress	V
86-3536	Left eye swab	Rule out adenovirus	V
86-3996	Conjunctiva	Rule out adenovirus	V
86-3969	NP	Pharyngitis	V
86-2281	NP	Pneumonia	V
86-3681	Throat	Pancytopenia	V
86-1823	Stool	Fever of unknown origin	V
86-1869	Throat		V
86-1041	Throat		V

[a] Restriction patterns are designated by letters or Roman numerals according to the nomenclature of Bailey and Richmond (2) except in the cases of *Pst*I and *Hin*dIII digestions in which new patterns were observed. The restriction patterns for *Bam*HI, *Pst*I, and *Sma*I digestions for all isolates are 3a, E, and III, respectively.

[b] NP, Nasopharyngeal.

[c] Catheter specimen.

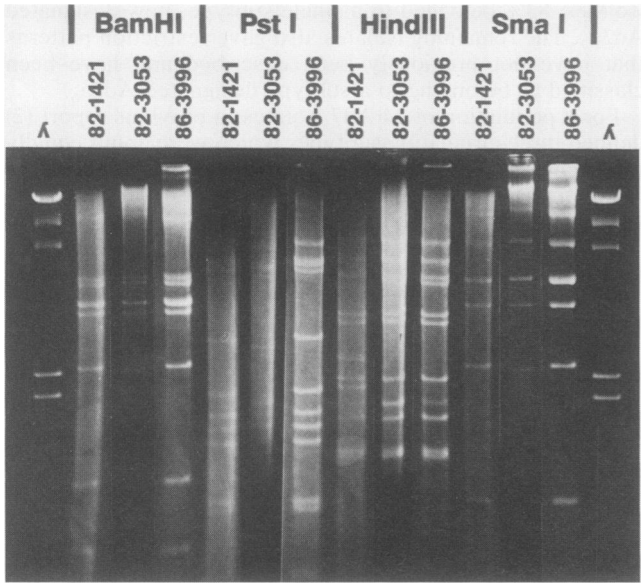

FIG. 2. Agarose gel electrophoresis of DNA fragments from restriction enzyme digestion of representative Ad3a isolates. Partially purified DNAs were digested with restriction enzymes, and fragments were subjected to electrophoresis in 1.2% agarose containing ethidium bromide and photographed under UV light. Lambda DNA fragments are 23.1, 9.6, 6.3, 4 (not visible), 2.3, and 2.1 kilobase pairs in size.

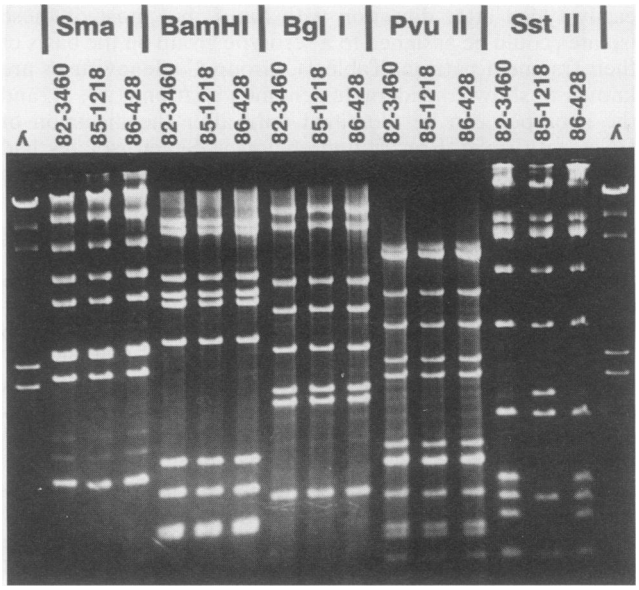

FIG. 3. Agarose gel electrophoresis of DNA fragments from restriction enzyme digestion of representative Ad7b isolates. DNA was digested, subjected to electrophoresis in agarose, and photographed under UV light. Lambda DNA fragments are described in the legend to Fig. 2. Faint partial digestion products are visible in some lanes.

TABLE 3. Ad7b isolates from 1981 to 1986

Isolate no.	Specimen	Diagnosis	*Sst*II restriction pattern[a]
81-263	Tracheal aspirate	Bilateral pneumonia	III
81-450	Tracheal aspirate	Pneumonia	III
81-340	Throat	Failure to thrive	III
82-1166	Eye	Conjunctivitis	III
82-1494	Eye drainage	Eye drainage	III
82-1495	NP[b]	Rule out sepsis	III
82-1513	Stool	Rule out sepsis	III
82-1870	Eye	Eye infection	III
82-2342	Throat	Asthma	III
82-2925	NP	Rule out sepsis	III
82-3451	Stool	Gastroenteritis	III
82-3448	Tracheal aspirate	Gastroenteritis	III
82-3460	NP	Gastrointestinal bleeding	III
82-3462	NP	Pneumonia	III
82-3559	NP	Apnea	III
82-3682	NP	Rule out conjunctivitis	III
83-2746	Urine	Seizures (mumps)	III
83-4190	Eye drainage	Conjunctivitis	III
84-4039	Bronchial wash	Pneumonia	III
84-4044	Conjunctiva	Conjunctivitis	III
85-3393	Conjunctiva swab	Conjunctivitis	III
85-3266	Stool		III
85-3247	Throat		III
85-3224	Eye	Conjunctivitis	III
85-3095	Rectal	Gastroenteritis	III
85-2905	Eye swab	Diarrhea and conjunctivitis	III
85-2157	Rectal	Gastroenteritis	III
85-1218	NP or throat or both	Pneumonia	IV
85-19	Eye drainage	Conjunctivitis	III
86-332	Throat		III
86-418	NP	Pneumonia; rule out RSV[c]	III
86-428	Mucous sputum	Pneumonia; RSV	III
86-750	Eye	Eye infection	III
86-864	Eye	Rule out adenovirus	III
86-3337	Eye	Conjunctivitis	III
86-2485	Eye	Rule out adenovirus	III
86-3193	Eye	Rule out adenovirus	III
86-3188	Left eye	Rule out adenovirus	III
86-4138	Throat	Pneumonia	III
86-4091	Left eye	Rule out adenovirus	III
86-4087	Right conjunctiva	Rule out adenovirus	III
86-4758	Left eye	Rule out adenovirus	III
86-6022	Throat		III
86-6797	Eye	Periorbital cellulitis	III

[a] Restriction patterns are designated by letters or Roman numerals according to the nomenclature of Bailey and Richmond (2). The restriction patterns for *Bam*HI, *Bgl*I, and *Pvu*II digestions for all isolates are 7b, B, and Y, respectively.
[b] NP, Nasopharyngeal.
[c] RSV, Respiratory syncytial virus.

ular epidemiologists. Wadell et al. (8) reported that between 1958 and 1969 the predominant Ad7 genome type in Europe was Ad7c. This was replaced by Ad7b as the predominant species from 1970 to the early 1980s. Bailey and Richmond (2) confirmed Ad7b as the major species in Manchester, England, during a 16-month epidemic from 1983 to 1985 and further established the almost equal distribution of the three genome subtypes. In the United States between 1962 and 1980, the predominant strain was Ad7b which made up about 65% of the isolates reported by Wadell (8). Ad7a made up about 24% of the U.S. Ad7 isolates during the same period. Ad7b has recently become the predominant Ad7 species in Australia but has been reported only rarely in Africa and not at all in China, Japan, or South America.

The global, as well as local, distribution of various adenovirus subtypes presents interesting epidemiological problems which merit continuing investigation. The current evidence does not support the theory that some subtypes are more virulent than others, but the possibility exists that new emerging subtypes might cause more severe disease than the ones they supplant. The genetic stability of subtypes is

TABLE 4. Summary of Ad3 and Ad7 genome subtypes isolated from 1981 to 1986 in St. Louis, Mo.

Genome subtype[a]	No. of isolates (%)
$Ad3a_6$	4 (16.7)
$Ad3a_7$	20 (83.3)
$Ad7b_1$	1 (2.3)
$Ad7b_2$	43 (97.7)

[a] These designations are according to the nomenclature of Bailey and Richmond (2), who defined the restriction enzyme profiles for subtypes Ad3a through $Ad3a_5$ and for subtypes Ad7b through $Ad7b_3$.

unknown, and the ease and speed with which new subtypes replace old ones are also unknown. The worldwide heterogeneity of adenovirus subtypes is slowly becoming understood, but the epidemiology in local microenvironments is apparently poorly resolved and deserves further analysis.

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