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Sensitive Analysis of Genetic Heterogeneity of Adenovirus Types 3 and 7 in the Soviet Union

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An analysis of adenovirus strains isolated in the Soviet Union from 1976 to 1988 revealed four genome types of adenovirus type 3 (Ad3), i.e., Ad3a4, Ad3a9, Ad3a10, and Ad3a11, and four genome types of adenovirus type 7 (Ad7), i.e., Ad7p, Ad7a, Ad7a(1-5), and Ad7f1, identified with the DNA restriction enzymes *Bam*HI, *Bgl*II, and *Hin*dIII. Three of them, Ad3a10, Ad3a11, and Ad7f1, are newly discovered. The genetic heterogeneity of adenoviruses was examined with restriction endonuclease *Cfr*13I with a 4-base recognition cleavage site. Eighteen different restriction patterns were identified among 21 selected Ad3 strains after cleavage of DNA with *Cfr*13I. Eight different subtypes were identified among 20 Ad7 strains by the same technique. For estimation of the relationships among these genome subtypes, pairwise analyses of comigrating DNA restriction fragments from isolates of Ad3 and Ad7 were done after digestion with *Cfr*13I or with restriction endonucleases recognizing DNA sequences of 6 bp. Surprisingly, the results were very discrepant.

Adenovirus types 3 (Ad3) and 7 (Ad7) are most frequently isolated as epidemic causes of severe respiratory infections, especially in children (16). Ad3 and Ad7 show significant genetic variability (1, 2, 4, 5, 9, 10, 13, 17).

Restriction endonucleases (REs) are widely used to investigate these genetic diversities. Thirty-one different genome types of Ad3 (2, 10) and 20 different genome types of Ad7 (1, 9) have been previously identified with REs with 6-base recognition cleavage sites.

In this study, we have analyzed 21 strains of Ad3 and 20 strains of Ad7 with REs with 6- and 4-base recognition cleavage sites to confirm the advantages of using REs with 4-base recognition cleavage sites. However, we have obtained contradictory results, which are discussed.

MATERIALS AND METHODS

Virus strains. Twenty-one strains of Ad3 and 20 strains of Ad7 recovered from different regions of the Soviet Union from 1976 to 1988 were selected from the All-Union Collection of Influenza and Respiratory Viruses, Leningrad, USSR. A large majority of the adenovirus strains was collected from patients with respiratory diseases. To retrace evolutionary relationships, we selected strains from limited sources over the longest possible period. Types of adenoviruses were identified by neutralization tests with specific antisera obtained from the All-Union Research Institute of Influenza by immunization of rabbits with Ad3 and Ad7 reference strains kindly provided from the International Collection of Virus Strains, Bethesda, Md.

Preparation of viral DNA. After one or two passages with terminal dilutions in human embryo kidney cell cultures, the viruses were propagated in monolayers of HeLa or HEp-2 cells from the Collection of Cell Cultures of the All-Union Research Institute of Influenza. Infected cells were incubated in Eagle minimal essential medium (Institute of Epidemiology and Microbiology, Moscow, USSR) with a double concentration of arginine until cytopathic effects were almost complete. DNA was prepared from complete virions purified by cesium gradient centrifugation as described elsewhere (14).

DNA restriction. The enzymes *Bam*HI, *Bgl*II, *Hin*dIII, *Cfr*13I, and *Sau*3AI were purchased from Ferment, Vilnius, Lithuania. The treatment was carried out in a total volume of 20 μl in accordance with the instructions of the manufacturer.

Labeling and purification of DNA restriction fragments. After digestion with REs, DNA restriction fragments were labeled at the 3′ terminus with the *Escherichia coli* DNA polymerase I Klenow fragment (Ferment) as described elsewhere (6). [α-^{32}P]dCTP (All-Union Association Isotop, Leningrad, USSR) and [α-^{35}S]dCTP (Amersham) were used for labeling, and restriction fragments with protruding single-stranded 5′ ends were analyzed. After labeling was done, DNA was separated from the unincorporated nucleotides by centrifugation through Sephadex G-25 Fine (15).

Agarose gel electrophoresis of DNA restriction fragments. Adenovirus DNAs digested with *Bam*HI, *Bgl*II, and *Hin*dIII were labeled, purified, and loaded on 1.0% agarose gels (type I-A; Sigma) with Tris-acetate electrophoresis buffer (2 mM EDTA, 20 mM sodium acetate, 40 mM Tris [pH 7.8]). Horizontal electrophoresis was done at 2.5 V/cm for 15 h. Agarose gels were soaked in 60% ethanol for 2 h, dried on aluminum foil plates at 37°C, and exposed to HS 11 X-ray film (ORWO). λ phage DNAs digested with *Hin*dIII were used as the size markers for DNA fragments.

Polyacrylamide gel electrophoresis. Adenovirus DNAs digested with *Cfr*13I were labeled, purified, precipitated by the addition of 4 volumes of acetone containing 2% $LiClO_4$ for 5 min at room temperature, and centrifuged at 5,000 × *g* for 5 min. The sediments were dissolved in 2 μl of loading buffer (15% glycerol, 0.1% xylene cyanol FF, 0.1% bromophenol blue, 10 mM EDTA [pH 8.0]) and displayed on 5.7% polyacrylamide (acrylamide–*N*,*N*′-methylenebisacrylamide ratio, 6:1) slab (450 by 200 by 0.3 mm) gels (11). This very high proportion of *N*,*N*′-methylenebisacrylamide seems to suppress the effect of sequence on mobility, allowing the resolution of mixed-sequence DNA fragments from 8 to

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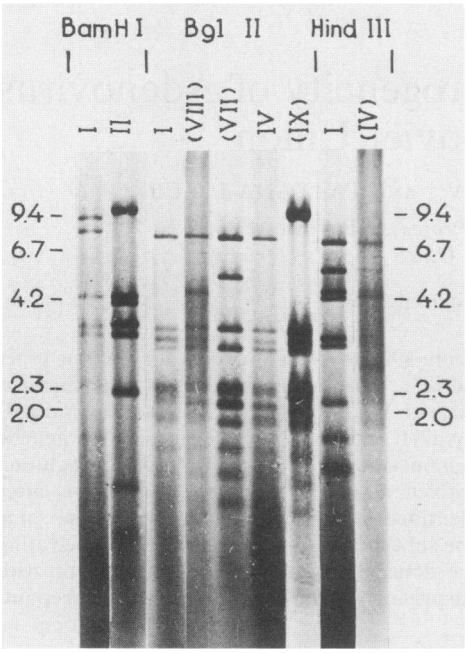

FIG. 1. DNA cleavage patterns of Ad3 variants with the indicated enzymes. Restriction patterns are labeled in accordance with the nomenclature of Li and Wadell (10); newly discovered restriction patterns are in parentheses. The numbers on the left and on the right indicate the positions and sizes of the *Hin*dIII fragments of bacteriophage λ on this gel.

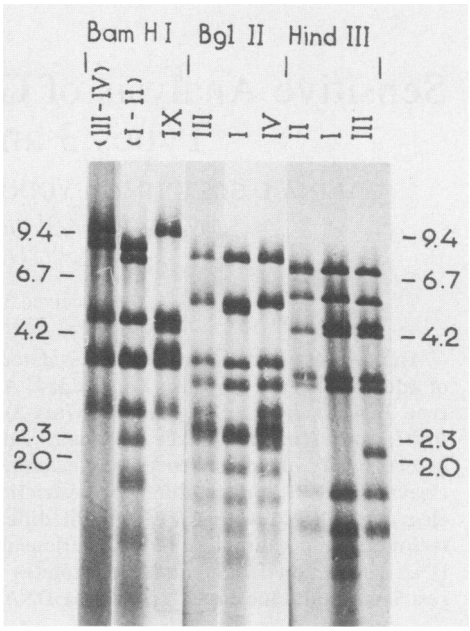

FIG. 2. DNA cleavage patterns of Ad7 variants with the indicated enzymes. Restriction patterns are labeled in accordance with the nomenclature of Li and Wadell (9); the III-IV and I-II restriction patterns could not be distinguished under the conditions used. The numbers on the left and on the right indicate the positions and sizes of the *Hin*dIII fragments of bacteriophage λ on this gel.

2,000 bp, approximated to the nearest integers. Although the absolute sizes of large DNA fragments can be defined, but with some error, this error is markedly reduced in the evaluation of relative displacements of some fragments close in size. A gradient buffer in the gel was established from 0.4× to 3.2× TPB (1× TPB is 2 mM EDTA plus 80 mM Tris-phosphate [pH 8.15]). Prerunning for 0.5 h and running for about 5 h were done at 10 V/cm. Polyacrylamide gels on glass plates were soaked sequentially for 5 min each time in 10% acetic acid, water, and ethanol, dried at 80°C for 1 h, and exposed to HS 11 X-ray film. Plasmid pBR322 DNAs digested with *Sau*3AI were used as size markers for DNA fragments.

RESULTS

Identification of Ad3 and Ad7 genome types. The analysis of DNA from Ad3 and Ad7 isolates was carried out with REs with 6-base recognition cleavage sites, i.e., *Bam*HI, *Bgl*II, and *Hin*dIII. The DNA restriction patterns of the different adenovirus genome types are shown in Fig. 1 and 2. The genome types of selected adenoviruses are shown in Table 1. Newly discovered adenovirus genome types were noted as an extension to the nomenclature of Li and Wadell (9, 10).

Four genome types of Ad3 were found in the Soviet Union. The DNA restriction patterns observed after digestion of DNA from the Ad3a10 and Ad3a11 genome types with *Bgl*II were new. The DNA restriction pattern observed after digestion of DNA from the Ad3a10 genome type with *Hin*dIII was also new. Therefore, adenoviruses of the Ad3a10 and Ad3a11 genome types were isolated exclusively in the Soviet Union.

Ad3a10 (Voronezh, 1979; Khabarovsk, 1979) and Ad3a11 (Voronezh, 1982) were identified in sporadic cases. Isolates with the genome types Ad3a4 and Ad3a9 were recovered from 1978 to 1988 and from 1979 to 1985, respectively.

Four genome types of 20 Ad7 isolates were identified in the Soviet Union. Ad7a predominated from 1976 to 1979 in the European region of the USSR (Dnepropetrovsk, Donetsk, and Voronezh). Ad7p (Voronezh, 1980; Arkhangelsk, 1981) and Ad7a(1-5) (Minsk, 1976; Donetsk, 1980) were identified in sporadic cases. Adenoviruses exclusively of the Ad7f1 genome type were isolated in many regions (Mogilyov, Donetsk, Kherson, and Alma-Ata) from 1986 to 1988. This genome type was new and considered to be the predominant one. Ad7f1 DNA digested with *Bam*HI and

TABLE 1. Cleavage patterns obtained when 21 Ad3 and 20 Ad7 strains were digested with *Bam*HI, *Bgl*II, and *Hin*dIII

Genome type[a]	Profile[b] with:			No. (%) of strains
	*Bam*HI	*Bgl*II	*Hin*dIII	
Ad3p (reference)	I	I	I	
Ad3a4	II	IV	I	12 (56)
Ad3a9	II	(VII)	I	6 (29)
Ad3a10	II	(VIII)	(IV)	2 (10)
Ad3a11	II	(IX)	I	1 (5)
Ad7a (reference)	III-IV	III	II	
Ad7a	III-IV	III	II	7 (35)
Ad7a(1-5)[c]	III-IV	III	III	3 (15)
Ad7f1	IX	IV	III	8 (40)
Ad7p	I-II	I	I	2 (10)

[a] Genome types are in accordance with the nomenclature of Li and Wadell (9, 10).
[b] Corresponding to those shown in Fig. 1 and 2.
[c] Contains a1, a3, a4, and a5 genome types; detailed genome typing has not been done.

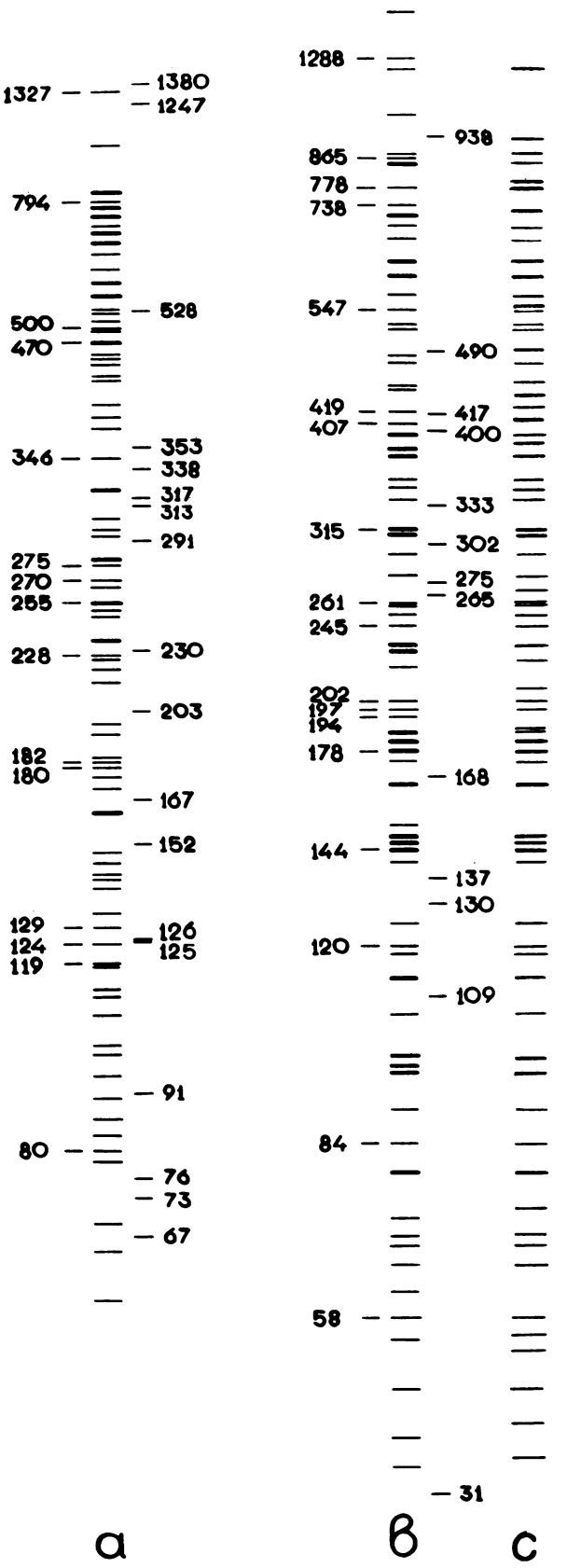

FIG. 3. Schematic representation of restriction patterns obtained after cleavage of DNA from Ad3a4/Donetsk/3533/87 (a), Ad7a/Dnepropetrovsk/542/78 (b), and Ad7p/Voronezh/1708/80 (c) with RE *Cfr*13I. The numbers on the left indicate the positions and sizes of the different DNA restriction fragments belonging to the above-mentioned strains. The numbers on the right indicate the positions and sizes of the different DNA restriction fragments belonging to other strains of Ad3 (Table 2) and Ad7 (Table 4).

*Hin*dIII but not *Bgl*II manifested the same DNA restriction patterns as did the Ad7f genome type described by Li and Wadell (9). The DNA restriction pattern of Ad7f1 digested with *Bgl*II was similar to that of Ad7a(1-5) but not Ad7f.

DNA analysis with RE *Cfr*13I with a 4-base recognition cleavage site. Detailed identification of adenovirus genome types was done with RE *Cfr*13I recognizing the 4-base cleavage site G'GNCC. A schematic representation of restriction patterns obtained after cleavage of DNA from different adenovirus genome types with *Cfr*13I is shown in Fig. 3.

(i) **Ad3.** DNAs of 21 Ad3 strains were analyzed with *Cfr*13I, and 74 to 81 DNA fragments were revealed. Eighteen subtypes within four genome types (Ad3a4, Ad3a9, Ad3a10, and Ad3a11) were identified, indicating the great genetic heterogeneity of Ad3 strains. A comparison of DNA restriction patterns identified 32 different DNA fragments (Table 2). The strains were grouped in such a way as to emphasize the restriction pattern homologies among them. Genetic heterogeneity was shown to be connected with 361- to 228-bp DNA fragments, while Ad3 genome types were determined by 1,380- to 470-bp and by 204- to 67-bp DNA fragments. Thus, fragments of 1,380 and 1,247 bp were observed but fragments of 1,327 and 794 bp were never observed exclusively in the DNA restriction pattern of Ad3a11. The Ad3a10 genome type was characterized by the detection of a 502-bp DNA fragment instead of a 500-bp DNA fragment, by the manifestation of a cluster of fragments of 167, 152, 126, 125, 91, 76, and 73 bp, and by the lack of a cluster of fragments of 129, 124, 119, and 80 bp. A 470-bp DNA restriction fragment was absent in five of six Ad3a9 strains, with one exception, strain Voronezh/1742/81.

Two DNA fragments (ca. 255 and 230 bp) were present in slightly different sizes in eight combinations. However, the combinations of 255 and 230 bp, 255 and 228 bp, and null and 228 bp were retained for a time, while the other combinations were incidental. Some geographical relationships of adenovirus strains were reflected in such combinations, e.g., in the group of adenoviruses Donetsk/1522/79, Donetsk/2993/86, and Donetsk/3533/87 and in the group of adenoviruses Khabarovsk/1802/80, Khabarovsk/3272/81, and Khabarovsk/2956/85.

To estimate the genetic relationships among Ad3 strains, we carried out a pairwise analysis of comigrating DNA restriction fragments (Table 3). We identified 11 different subtypes among 12 strains of the Ad3a4 genome type and 4 different subtypes among 6 strains of the Ad3a9 genome type. The degree of pairwise comigrating restriction fragments (PCRFs; 4-PCRFs when the RE with a 4-base recognition cleavage site was used and 6-PCRFs when the REs with 6-base recognition cleavage sites were used) varied from 83 to 100%.

No identical DNA restriction patterns of Ad3 strains were observed in different areas over 1 year, while the circulation of closely related subtypes occurred for a time in the same areas. Thus, in Donetsk from 1986 to 1987, Ad3a4 strains displaying 100% 4-PCRFs were identified, and in Khabarovsk, strains with identical subtypes of the Ad3a9 genome type were isolated from 1980 to 1985 (Fig. 4).

TABLE 2. Sizes of different Ad3 DNA restriction fragments obtained with RE *Cfr*13I

Isolate	Genome type[a]	Total no. of fragments	Presence (+) or absence (−) of a DNA restriction fragment of the indicated size (bp)[b]:																																
			1,380	1,327	1,247	794	528	500	470	353	346	338	317	313	291	275	270	255	230	203	182	180	167	152	129	126	125	124	119	91	80	76	73	67
Lutsk/1536/80	a4	77	−	+	−	+	−	+	+	−	+	−	−1	+	−	+2	+	+3	−2	−	+	+	−	−	−	+	+	−	−	−	+	−	−	−
Saratov/1178/79	a9	76	−	+	−	+	−	+	−	−	+	−	−1	+	−	+2	+	+3	+	−	+	+	−	−	−	+	+	−	−	−	+	−	−	−
Donetsk/1643/80	a9	76	−	+	−	+	−	+	−	−	+	−	−	+	+	−	+	−	−2	+	+	+	+	−	−	+	+	−	−	−	+	−	−	−
Khabarovsk/1802/80	a9	75	−	+	−	+	−	+	−	−	+	−	+	−	−	+	+	−	−2	−	−1	−2	−	−	+	−	−	+	+	−	+	−	−	−
Khabarovsk/3272/81	a9	75	−	+	−	+	−	+	−	−	+	−	+	−	−	+	+	−	−2	−	−1	−2	−	−	+	−	−	+	+	−	+	−	−	−
Khabarovsk/2956/85	a9	75	−	+	−	+	−	+	−	−	+	−	+	−	−	+	+	−	−2	−	−1	−2	−	−	+	−	−	+	+	−	+	−	−	−
Donetsk/1752/81	a4	77	−	+	−	+	−	+	+	−	+	−	+	−	−	+	+	+	+	−	+	+	−	−	+	−	−	+	+	−	+	−	−	−
Voronezh/2174/82	a11	77	+	−	+	−	−	+	+	−	+	−	+	−	−	+	+	+	+	−	−1	−2	−	−	+	−	−	+	+	−	+	−	−	−
Voronezh/1134/79	a10	81	−	+	−	+	−	+2	+	+8	+	−	+	−	−	+2	−	+	+	+1	+	+	+	+	−	+	+	−	−	+	−	+	+	−
Kherson/2649/84	a4	77	−	+	−	+	−	+	+	−	+	−	+	+	−	+	+	+	+	−	+	+	−	−	−	+	+	−	−	−	+	−	−	−
Khabarovsk/920/78	a4	77	−	+	−	+	+	+	+	−	+	+	−	−	−	−	+	+	+	−	−2	−2	−	−	+	−	−	+	+	−	+	−	−	−
Khabarovsk/1086/79	a10	79	−	+	−	+	−	+2	+	+	+	−	−	−	−	−	−	+	+	−	+	+	+	+	−	+	+	−	−	+	−	+	+	+
Arkhangelsk/501/78	a4	79	−	+	−	+	−	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	−	−	+	+	−	−	−	+	−	−	−
Arkhangelsk/1510/79	a4	79	−	+	−	+	−	+	+	+	+	−	−	−	+	+	+	+6	−2	+	+	+	+	−	−	+	+	−	−	−	+	−	−	−
Khabarovsk/1810/81	a4	75	−	+	−	+	−	+	+	−	+	−	−	−	−	−	+	+6	−2	−	+	+	−	−	+	−	−	+	+	−	+	−	−	−
Donetsk/3639/88	a4	75	−	+	−	+	−	+	+	−	+	−	−	−	−	−	+	+2	+1	−	+	+	−	−	+	−	−	+	+	−	+	−	−	−
Voronezh/1742/81	a9	74	−	+	−	+	−	+	+	−	+	−	−	−	−	−	+	+2	−1	−	+	+	−	−	−	−	−	+	+	−	+	−	−	−
Donetsk/2993/86	a4	76	−	+	−	+	−	+	+	−	+	−	−	−	−	+	+	+	−2	−	+	+	−	−	+	−	−	+	+	−	+	−	−	−
Donetsk/3533/87	a4	76	−	+	−	+	−	+	+	−	+	−	−	−	−	+	+	+	−2	−	+	+	−	−	+	−	−	+	+	−	+	−	−	−
Donetsk/1522/79	a4	79	−	+	−	+	−	+	+	−	+	+	−	+2	+	−	+	+	−2	+	+	+	+	−	−	+	+	−	−	−	+	−	−	−
Arkhangelsk/2216/82	a4	76	−	+	−	+	−	+	+	−	−6	−	−	−	+	−	+	+3	+	−	+	+	+	−	−	+	+	−	−	−	+	−	−	−

[a] Determined with REs *Bam*HI, *Bgl*II, and *Hin*dIII.

[b] A number following a symbol indicates a DNA fragment with an altered size; e.g., −1 means a DNA fragment of 317 − 1 = 316 (bp).

FIG. 4. Schematic maps of the relationships among Ad3 genome variants. The diagrams were drawn with REs recognizing DNA sequences of 6 bp (a) and of 4 bp for Donetsk (b) and Khabarovsk (c). The numbers are percentages of comigrating fragments. Degrees of homology in PCRFs are indicated as follows: ▬, highest; ——, second highest; ---, highest or second highest below 90%.

TABLE 3. Percentages of 4-PCRFs of Ad3 genome types obtained from cleavage with RE *Cfr*13I

Isolate	Genome type[a]	% 4-PCRF with:																					
		Donetsk							Khabarovsk					Voronezh			Arkhangelsk					
		Kherson 2649/84 (a4)	Lutsk 1536/80 (a4)	Saratov 1178/79 (a9)	1522/79 (a4)	1643/80 (a9)	1752/81 (a4)	2993/86 (a4)	3533/87 (a4)	3639/88 (a4)	920/78 (a4)	1802/80 (a9)	3272/81 (a9)	2956/85 (a9)	1086/79 (a10)	1810/81 (a4)	1742/81 (a9)	2174/82 (a11)	1134/79 (a10)	1510/79 (a4)	2216/82 (a4)
501/78[b]	a4	96	92	93	96	96	94	93	93	91	90	88	88	88	92	91	92	89	90	98	96
2649/84	a4		95	95	94	94	96	94	94	92	91	91	91	91	91	92	93	91	91	94	94
1536/80	a4			98	94	95	91	93	93	92	88	91	91	91	89	93	93	86	89	94	94
1178/79	a9				92	92	92	91	91	91	89	89	89	89	89	91	92	86	89	92	95
1522/79	a4					97	91	93	93	91	90	89	88	88	90	92	92	86	89	96	94
1643/80	a9						90	92	92	91	89	90	90	90	89	93	92	85	88	97	95
1752/81	a4							98	98	96	95	95	95	95	89	96	95	95	89	91	92
2993/86	a4									97	94	95	95	95	88	98	96	93	87	93	92
3639/88	a4										93	93	93	93	87	97	98	91	86	91	91
920/78	a4											95	95	95	86	93	93	95	85	87	89
1802/80	a9														83	95	93	95	83	90	87
1086/79	a10															87	88	83	96	90	90
1810/81	a4																97	91	86	94	91
1742/81	a9																	90	87	92	92
2174/82	a11																		84	86	86
1134/79	a10																			88	89
1510/79	a4																				94

[a] Determined with REs *Bam*HI, *Bgl*II, and *Hin*dIII.
[b] Ad3/Arkhangelsk/501/78.

Moreover, some strains of the Ad3a9 genome type from the European region of the USSR displayed more significant genetic relatedness with some strains of Ad3a4 than with those of Ad3a9 (Table 3); e.g., Ad3a4/Donetsk/3639/88 and Ad3a9/Voronezh/1742/81 were very closely related and displayed 98% 4-PCRFs, two outbreak strains, Ad3a4/Lutsk/1536/80 and Ad3a9/Saratov/1178/79, also displayed 98% 4-PCRFs, and Ad3a9/Voronezh/1742/81 and Ad3a9/Donetsk/1643/80 displayed only 92% 4-PCRFs.

The Ad3 subtypes displayed in Table 3 were grouped according to the highest pairwise homology, at least 96% (Fig. 5). This grouping corresponded to some limited sources or defined periods. An analysis of relationships among the groups revealed three group clusters with 90 to 95% pairwise homology of adenovirus genomes within any cluster: the first cluster included the adenovirus subtypes recovered from 1978 to 1982 from the European region of the USSR (Arkhangelsk, Donetsk, Lutsk, and Saratov), the second cluster included strains of the Ad3a10 genome type recovered from 1978 to 1985 from the Asian region of the USSR (Khabarovsk) and from 1981 to 1988 from the European region of the USSR (Donetsk and Voronezh).

(ii) Ad7. DNAs of 20 Ad7 strains were analyzed with

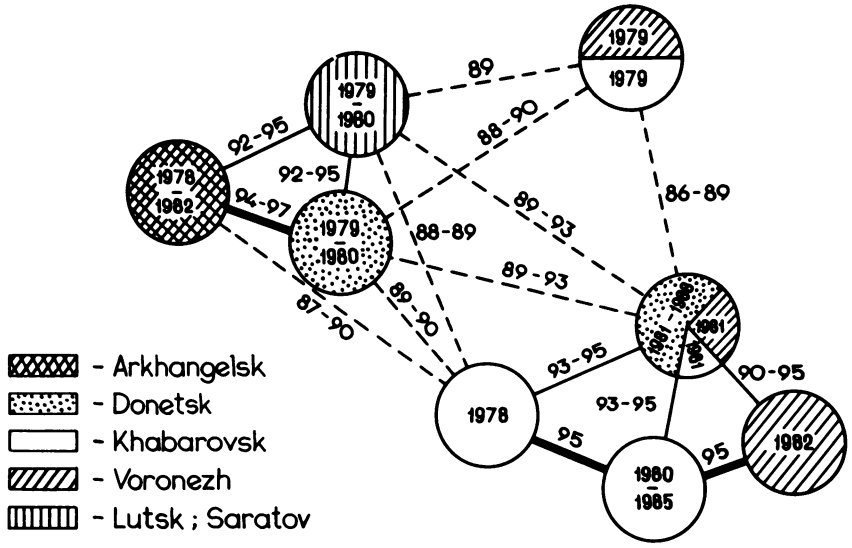

FIG. 5. Schematic maps of the relationships among Ad3 subtypes. The diagram was drawn with RE *Cfr*13I recognizing DNA sequences of 4 bp. The numbers are percentages of comigrating fragments. Degrees of homology in 4-PCRFs are indicated as follows: ▬, highest within one cluster; ——, second highest within one cluster; ---, highest or second highest between different clusters.

*Cfr*13I, and 70 to 79 DNA fragments were revealed; 43 different DNA fragments were identified.

Two strains of the Ad7p genome type, Voronezh/1708/80 and Arkhangelsk/1825/81, were found. There were no differences in the DNA restriction patterns between these strains. Ad7p and the group of genome types including Ad7a, Ad7a(1-5), and Ad7f1 were very different, as determined by DNA restriction analysis with *Cfr*13I. The DNA restriction patterns of Ad7a, Ad7a(1-5), and Ad7f1 were analyzed, and eight subtypes with 30 different DNA fragments were identified (Table 4).

Strains of the Ad7f1 genome type showed genetic uniformity. Only Kherson/3022/86 displayed some differences in DNA restriction patterns from the other seven Ad7f1 strains; i.e., an 84-bp DNA restriction fragment was absent in the DNA profile of this strain.

The greatest genetic heterogeneity was connected with the Ad7a genome type. Eight strains of the Ad7a genome type, including the reference strain, were classified into four different subtypes. However, four strains of the Ad7a11 subtype with identical restriction patterns were isolated in 1977, and lethal outcomes were observed for all carriers. Thereafter, the greatest genetic heterogeneity of Ad7 was determined by 1,288- to 538-bp and by 315- to 31-bp DNA fragments.

To estimate the genetic relationships among Ad7 subtypes, we carried out a pairwise analysis of comigrating restriction fragments of DNA digested with *Cfr*13I (4-PCRFs) (Table 5). Ad7 genome types were also analyzed with REs with 6-base recognition cleavage sites (6-PCRFs), i.e., *Bam*HI, *Bgl*II, and *Hin*dIII, for comparison with the 4-PCRFs of Ad7 subtypes.

Great differences between the magnitudes of 4-PCRFs and 6-PCRFs for some adenovirus pairs were observed. Variants of the Ad7a genome type not distinguished by 6-PCRFs were found significantly different by 4-PCRFs; i.e., Ad7a I and Ad7a II displayed 84% 4-PCRFs, Ad7a II and Ad7a III displayed 86% 4-PCRFs, but Ad7a I and Ad7a III displayed 99% 4-PCRFs (Table 5).

Diagrams of genetic relationships for Ad7 isolates were drawn with 4-PCRFs and 6-PCRFs (Fig. 6).

DISCUSSION

The molecular epidemiology and global distribution of Ad3 and Ad7 genome types were described elsewhere (1, 2, 9, 10, 17). However, the circulation of Ad3 and Ad7 genome types in the Soviet Union was not investigated. We carried out such an investigation of adenovirus strains isolated in the Soviet Union from 1976 to 1988.

Genome typing was performed with the DNA REs *Bam*HI, *Bgl*II, and *Hin*dIII. These 3 REs were selected from 12 used elsewhere (1, 2, 9, 10) as the most useful for genome typing of adenoviruses. The 6-PCRFs obtained with these three REs proved to be in good agreement with the 6-PCRFs obtained with the 12 REs described by Li and Wadell (9).

Four genome types of Ad3, i.e., Ad3a4, Ad3a9, Ad3a10, and Ad3a11, were identified. Ad3a4 predominated from 1976 to 1988 in the Soviet Union; it predominated in 1983 in China. Ad3a9 was also isolated often. Ad3a9 is similar to D10 recovered in Georgia (United States) in 1977 (2). Ad3a10 and Ad3a11 were new and were identified in sporadic cases. Several genome types of the same type cocirculated simultaneously, e.g., Ad3a4, Ad3a9, and Ad3a10 in 1979 and Ad3a4 and Ad3a9 from 1980 to 1981.

Four genome types of Ad7, i.e., Ad7p, Ad7a, Ad7a(1-5), and Ad7f1, were identified by 6-PCRFs. The successive shift from Ad7a (1976 to 1979) to Ad7f1 (1986 to 1988) was observed in the Soviet Union with the appearance of some sporadic cases of the Ad7p genome type. The existence of the Ad7 genome type shift has been observed previously in Europe, Australia, and North America (9, 16, 17). The Ad7f1 genome type isolated exclusively in the Soviet Union is related to the Ad7f genome type recovered in Australia in 1980 (9), although there are some significant differences.

The considerable variability of Ad3 and Ad7 genome types was discovered with the help of RE *Cfr*13I with a 4-base recognition cleavage site. However, in contrast to the great

TABLE 4. Sizes of different Ad7 DNA restriction fragments obtained with RE *Cfr*13I

Isolate	Subtype[a]	Total no. of fragments	Presence (+) or absence (−) of a DNA restriction fragment of the indicated size (bp)[b]:																														
			1,288	938	865	778	738	547	490	419	417	400	333	315	302	274	265	261	245	202	197	194	178	168	144	137	130	120	109	84	58	31
Reference/1800	a I	73	+	−	+	+	+	+	−	+	−10	−	−	+	−	−	−	−	+	+	+	+	+	−	+	−	−	+	−	+	−	−
Donetsk/141/77 Voronezh/121/77 Dnepropetrovsk/287/77 Dnepropetrovsk/288/77	a II	77	+	−	+	−12	+15	−9	−	+	−10	−	−	−	+	+	−	+	+	−2	+2	−	−	+	−	+	+	−	+	+	+	+
Dnepropetrovsk/129/76 Dnepropetrovsk/542/78	a III	75	+	−	+	+	+	+	−	+	−10	−	−	+	−	−	−	+	+	+	+	+	+	−	+	−	−	+	−	+	+	−
Dnepropetrovsk/1262/79	a IV	77	+	−	+	−12	+15	+	−	+	−10	−	−	−	+	+	−	+	+	+	+	+	+	−	+	−	+	+	−	+	+	−
Minsk/59/76	a(1-5) I	79	+	+	−	+	+	+	+	+7	+	+	+	+	−	−	+	+	+	+	+	+	+	−	+	−	−	+	−	+	+	−
Donetsk/1595/80 Donetsk/1645/80	a(1-5) II	79	+	+	−	+	+	+	+	+7	+	+	−	−	+	+	−	+	+	−2	+2	−	−	+	−	+	+	−	+	+	+	+
Kherson/3024/86 Mogilyov/3116/86 Kherson/3160/86 Kherson/3161/86 Alma-Ata/3234/87 Donetsk/3322/87 Donetsk/3636/88	f1 I	71	−	+	−	+	+	+	−	+	−	−	−	+	−	−	−	+	−	+	+	+	+	−	+	−	−	+	−	+	−	−
Kherson/3022/86	f1 II	70	−	+	−	+	+	+	−	+	−	−	−	+	−	−	−	+	−	+	+	+	+	−	+	−	−	+	−	−	−	−

[a] a, a(1-5), and f1 are the genome types of Ad7 evaluated with *Bam*HI, *Bgl*II, and *Hin*dIII; I, II, III, and IV are the subtypes of Ad7 evaluated with *Cfr*13I.
[b] See Table 2, footnote *b*.

TABLE 5. Percentages of PCRFs of Ad7 genome types

Subtype[a]	% PCRF with[b]:							
	a I	a II	a III	a IV	a (1-5) I	a (1-5) II	fl I	fl II
p	80/72	80/63	80/75	80/71	76/78	76/69	72/76	72/75
a I		100/84	100/99	100/93	89/92	89/83	85/96	85/95
a II			100/86	100/91	89/80	89/91	85/81	85/80
a III				100/95	89/94	89/84	85/96	85/95
a IV					89/89	89/85	85/91	85/90
a (1-5) I						100/89	96/93	96/93
a (1-5) II							96/83	96/82
fl I								100/99

[a] See Table 4, footnote *a*.

[b] The value in the numerator indicates the percentage of 6-PCRFs evaluated with *Bam*HI, *Bgl*II, and *Hin*dIII, and the value in the denominator indicates the percentage of 4-PCRFs evaluated with *Cfr*13I.

heterogeneity and cocirculation activity of Ad3, Ad7 is notable for the homogeneity of its subtypes circulating over 1 year.

A comparison of 4-PCRFs with corresponding 6-PCRFs was done to determine the genetic relationships of adenoviruses. The diagrams for Donetsk and Khabarovsk are drawn as examples to emphasize the advantages of RE techniques with 4- rather than 6-base recognition cleavage sites (Fig. 4).

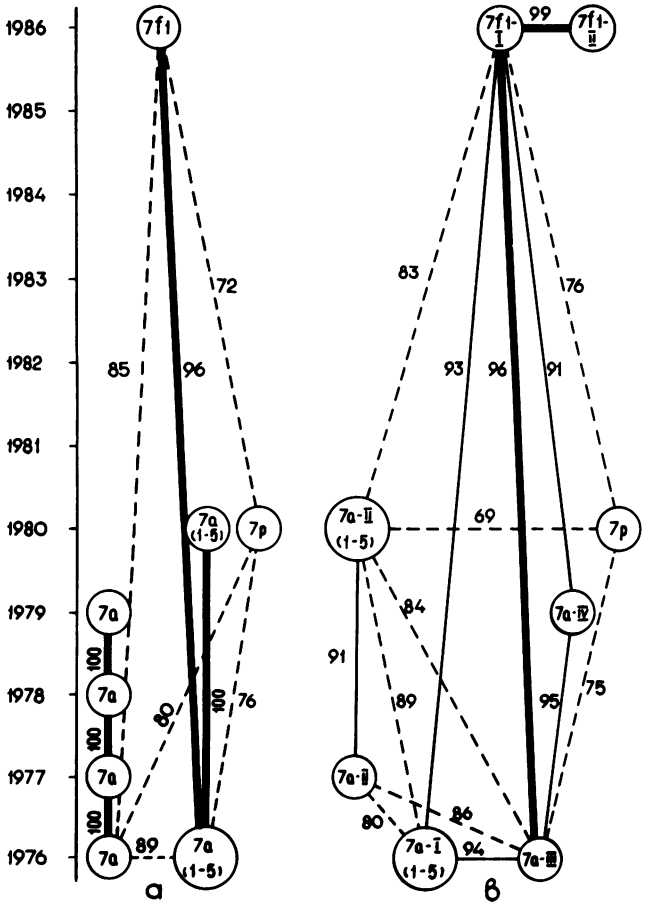

FIG. 6. Schematic maps of the relationships among Ad7 genome variants. The diagrams were drawn with REs recognizing DNA sequences of 6 bp (a) and of 4 bp (b). The numbers are percentages of comigrating fragments. Degrees of homology are shown as described the legend to Fig. 4.

The use of REs with 6-base recognition cleavage sites revealed the close genetic relationship and direct evolutionary correlation between Ad7fl and Ad7a(1-5) and the weak relationships between Ad7fl and Ad7a and between Ad7a and Ad7a(1-5) (Fig. 6). Surprisingly, the use of *Cfr*13I dramatically changed the evidence about the evolutionary relationships. Thus, the Ad7fl and Ad7a III subtypes were shown to be closely related, while the Ad7a(1-5) subtype was shown to be most closely related to the Ad7a II subtype. Therefore, the Ad7fl and Ad7a(1-5) II subtypes must be connected with different branches of Ad7a genome type evolution. Such an idea leads to the supposition of the existence of independent paths of Ad7 genome type evolution in the Soviet Union. The homology between Ad7a(1-5) subtype I and Ad7a(1-5) subtype II was shown to be only 89%.

An analysis of DNAs from Ad3 strains with *Cfr*13I revealed some trends in the evolution of Ad3; i.e., in 1981 and 1982 replacement of the first for the third cluster occurred among European Ad3 strains (Fig. 5).

Agarose gel electrophoresis done with REs recognizing DNA sequences of 6 bp determined nothing except a distribution of restriction sites in adenovirus DNAs that was of insufficient sensitivity to reveal the hot sites of adenovirus genome evolution. Polyacrylamide gel electrophoresis following digestion with *Cfr*13I revealed slight fragment alterations; that is why the results of tetranucleotide analysis are not equivalent to those of hexanucleotide analysis with 16 REs.

Thus, eight different combinations of two fixed DNA fragments with slight size alterations were observed among closely related Ad3 strains. These fragments, with sizes of about 255 and 230 bp, are supposed to be involved in site-specific genetic processes. Such an idea is not surprising, considering the recombination phenomena that occur between genomes of adenoviruses within one type (7), of different adenovirus types (12), and of adenovirus strains and host cells (8). Moreover, intermediate strains probably created by recombinations of adenovirus genomes have been recovered in nature (3, 10).

Tetranucleotide analysis of adenovirus DNAs is a suitable method for the detection of genome heterogeneities. It is more useful for determining accurate evolutionary relationships of adenoviruses than is hexanucleotide analysis. Hypervariable DNA restriction fragments among closely related genome types that may be of interest for subsequent mapping of hot sites on adenovirus genomes may be discovered.

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