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Analysis with Restriction Endonucleases Recognizing 4- or 5-Base-Pair Sequences of Human Adenovirus Type 3 Isolated from Ocular Diseases in Sapporo, Japan

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A total of 49 adenovirus type 3 (Ad3) strains from patients with epidemic keratoconjunctivitis at an eye clinic in Sapporo, Japan, from 1983 to 1986 were classified into either genome type Ad3f (26 strains) or Ad3g (23 strains). By the use of *Hin*fI and *Taq*I restriction endonucleases, they were classified into 11 and 3 subgenome types, respectively. During this observation period, two epidemics of Ad3 infection relating to epidemic keratoconjunctivitis occurred. The prevalent Ad3g strains isolated during the epidemic in 1983 belonged to a single subgenome type. However, in the course of the Ad3f epidemic in 1986, alteration of one subgenome type of the prevalent strain to a different subgenome type which was presumed to be a derivative of the former was observed. In the years in which only a few Ad3 strains were isolated, such Ad3f strains were classified into different subgenome types. However, all Ad3g strains except two belonged to the same subgenome type.

Human adenoviruses cause a variety of diseases, including respiratory, ophthalmic, gastrointestinal, and urinary tract infections (4, 5, 7, 8, 10–12, 15, 23, 24, 26–28, 30). Up to now, 47 adenovirus species have been recognized (13) and classified into six subgenera, A to F (28). Adenovirus type 3 (Ad3), belonging to subgenus B, has been demonstrated to be distributed all over the world (19). In Japan, Ad4 was the most important agent of epidemic keratoconjunctivitis caused by adenoviruses and accounted for 28% of the cases, while Ad3 was second most prevalent (24%) from 1983 to 1988, according to the report of the National Institute of Health in Japan (Monthly Report of Laboratory Findings of Infectious Agents, No. 10, 1988). Recently many genome types have been recognized in adenovirus strains by using restriction endonuclease (RE) analysis (1–3, 6, 8, 9, 11, 12, 14, 16, 18–22, 26, 27, 29–31), and this genome typing is an extremely useful tool in the field of epidemiology (28).

Among 49 genome types of Ad3 so far reported (1–3, 9, 11, 19, 21, 28), Ad3a, Ad3a3, Ad3c, Ad3-7, Ad3f, and Ad3g were isolated in Japan (11, 19). However, the prevalent genome types of Ad3 isolated recently in Japan were simple; only genome types Ad3f and Ad3g were identified in strains isolated in Japan (11). In this study, a total of 49 Ad3 strains isolated at an eye clinic in Sapporo from 1983 to 1986 were identified as simple genome type Ad3f or Ad3g. Therefore, it is difficult to judge by typing with REs recognizing 6-base-pair sequences whether these adenoviruses were really a homogeneous population or had been slightly changed in nucleotide sequence by changes too small to be detected by REs recognizing 6-base-pair sequences during the period. In order to classify genome types in more detail to monitor the alteration of prevalent strains in local microenvironments, we employed RE analysis with *Hin*fI and *Taq*I, which recognize 5- and 4-base-pair sequences, respectively, and cleave adenovirus DNA into many fragments, and examined the alteration of prevalent Ad3 strains in local microenvironments from an epidemiological point of view of the infection of adenoviruses.

All 49 Ad3 strains used here were isolated from the conjunctival swabs of local patients who attended one eye clinic in Sapporo during the period from 1983 through 1986. The genome types of 29 of these Ad3 strains have been reported by Guo et al. (11), and 20 strains were identified in this study. Viral DNA was extracted from the infected HeLa cells cultured in 150-mm-diameter petri dishes as described previously (25).

The REs used were obtained from Takara-Shuzo (Kyoto, Japan) and used under conditions described by the manufacturer. REs *Bam*HI, *Bgl*II, *Hin*dIII, and *Sma*I were used to identify genome types of Ad3 strains as described previously (11). To further classify the genome types, two REs, *Taq*I and *Hin*fI, which recognize tetra- and pentanucleotide sequences, were used, and resulting subclasses of genome types were provisionally called subgenome types. For classification of the subgenome type, the DNA fragments generated by digestion with *Taq*I and *Hin*fI enzymes were precipitated with 2.5 volumes of ethanol and then suspended in a buffer containing 2 mM EDTA, 4% 2-mercaptoethanol, 0.02% bromophenol blue, and 10% glycerol. Electrophoresis was performed according to a modification of the method of Laemmli (17) for protein samples, with 8 to ~10% polyacrylamide gels as running gels and 5% polyacrylamide gels as stacking gels at 13 mA for 10 h. Electrophoresis was carried out at 2°C. Gels were photographed after silver staining with a silver staining kit (Daiichi Pure Chemicals, Tokyo, Japan).

During the period from 1983 to 1986, 49 Ad3 strains were isolated, and 80% of the strains were obtained in 1983 (41%) and 1986 (39%). Outbreaks of Ad3 infection related to eye diseases seem to have occurred in both years in the area where the clinic is located. All 49 Ad3 strains were classified with *Bam*HI, *Bgl*II, *Hin*dIII, and *Sma*I into the types of Ad3f (26 strains) or Ad3g (23 strains) that were reported by Guo et al. (11). Both genome types were isolated every year during this observation period of 4 years. Ad3g was dominant in 1983, when 16 of the 20 Ad3 strains were Ad3g,

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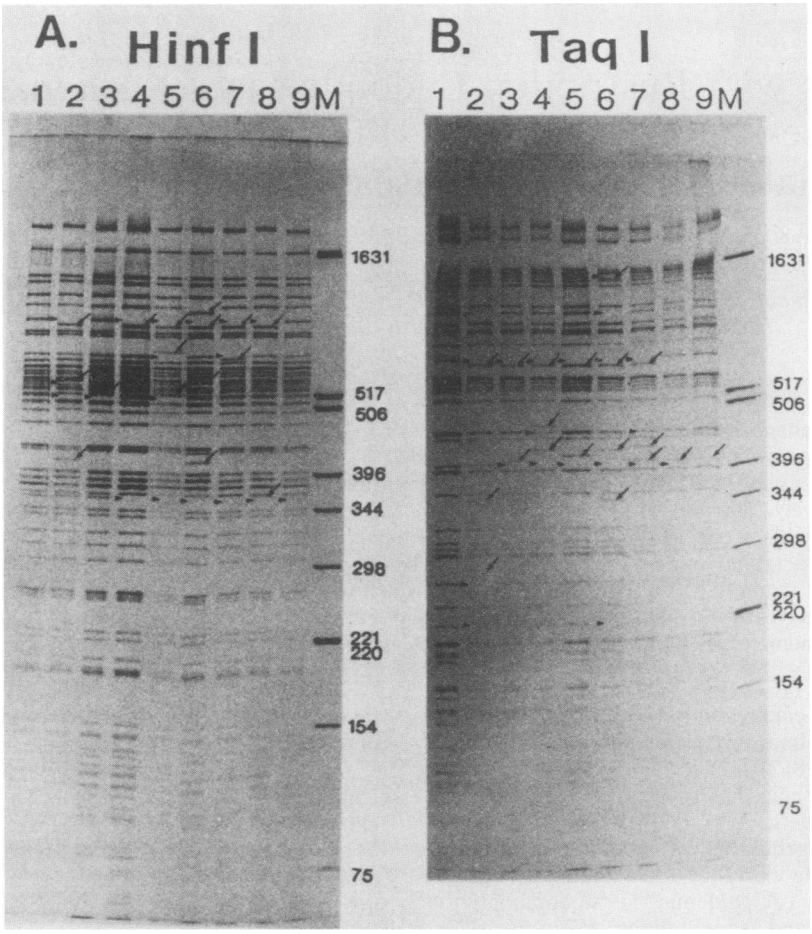

FIG. 1. DNA restriction patterns of Ad3f strains digested with *Hin*fI or *Taq*I. (A) Lane 1, TC2944 (Ad3fH1T1); lane 2, TC3155 (Ad3fH2T2); lane 3, TC3360 (Ad3fH3T1); lane 4, V3934 (Ad3fH4T3); lane 5, TC4496 (Ad3fH5T4); lane 6, TC4813 (Ad3fH6T6); lane 7, V5143 (Ad3fH7T7); lane 8, V5188 (Ad3fH8T3); lane 9, TC7205 (Ad3fH9T8). (B) Lane 1, TC2944 (Ad3fH1T1); lane 2, TC3155 (Ad3fH2T2); lane 3, V3934 (Ad3fH4T3); lane 4, TC4496 (Ad3fH5T4); lane 5, V4106 (Ad3fH4T5); lane 6, TC4813 (Ad3fH6T6); lane 7, V5143 (Ad3fH7T7); lane 8, TC7205 (Ad3fH9T8); lane 9, TC7678 (Ad3fH9T9). Lanes M, *Hin*fI-digested pBR322 with sizes marked in base pairs. Arrows and arrowheads point to additional and missing fragments, respectively, that were observed when the restriction patterns were compared with the prototype (lanes 1).

which accounted for 70% of the total 23 Ad3g strains, and in 1986, Ad3f was prevalent, with 16 of the 19 Ad3 strains being so identified. These 16 strains accounted for 62% of the 26 Ad3f strains found.

Among some REs examined, two REs, *Hin*fI and *Taq*I, cleaved Ad3 DNA into more than 60 fragments, of which more than 40 were between 200 and 1,700 base pairs long. Therefore, both enzymes were used in this experiment. Among the 26 strains of Ad3f, nine restriction patterns were able to be identified with each of the REs, and the patterns were numbered from 1 to 9 in chronological order of isolation of the strains (Fig. 1). Consequently, the 26 strains were classified into 11 subgenome types (Ad3fH1T1, Ad3fH2T2, Ad3fH3T1, Ad3fH4T3, Ad3fH5T4, Ad3fH4T5, Ad3fH6T6, Ad3fH7T7, Ad3fH8T3, Ad3fH9T8, and Ad3fH9T9) by the combination of both enzymes. Ad3fH1T1 was the dominant subgenome type (12 strains, 46%), and Ad3fH9T8 was next (5 strains, 19%).

Among the 23 strains of Ad3g, three and two restriction patterns were obtained with *Hin*fI and *Taq*I, respectively (Fig. 2), and three subgenome types (Ad3gH1T1, Ad3gH2T1, and Ad3gH3T2) were identified. Ad3gH1T1 was the dominant subgenome type (17 strains, 74%), and Ad3gH2T1 was next (5 strains, 22%).

Among subgenome types, one restriction pattern of a subgenome type was the same as that of the previously identified subgenome type but whose restriction pattern with one RE was different from that previously found with the other RE, as was the case with Ad3fH3T1 and Ad3fH1T1 or Ad3gH2T1 and Ad3gH1T1. We regarded the former as a possible derivative of the latter subgenome type, sharing a common restriction pattern with the enzyme.

In order to find chronological changes of the subgenome types, all strains of the respective genome types were arranged in order of isolation date (Table 1). In 1983, three subgenome types were identified in four strains, and two of them were Ad3fH1T1. One was Ad3fH3T1, which was distinguished from Ad3fH1T1 with *Hin*fI, and this strain may have derived from Ad3fH1T1. The remaining type was Ad3fH2T2, which had restriction patterns with both REs that were different from those of Ad3fH1T1 and Ad3fH3T1. All four strains isolated in 1984 were new subgenome types. However, it is conceivable that Ad3fH4T5 is a derivative of Ad3fH4T3. These subgenome types were identified only in

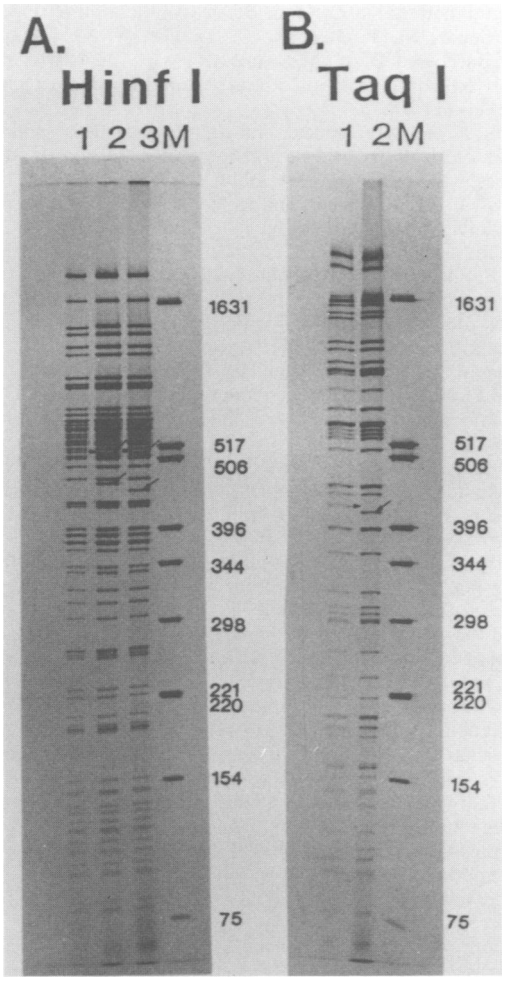

FIG. 2. DNA restriction patterns of Ad3g strains digested with *Hin*fI or *Taq*I. (A) Lane 1, TC3109 (Ad3gH1T1); lane 2, V5230 (Ad3gH2T1); lane 3, V5473 (Ad3gH3T2). (B) Lane 1, TC3109 (Ad3gH1T1); lane 2, V5473 (Ad3gH3T2). Lanes M, *Hin*fI-digested pBR322 with sizes marked in base pairs.

that year. In 1985, the two strains isolated were new subgenome types. One of the new types, Ad3fH8T3, seemed to be a possible derivative of Ad3fH4T3, isolated in 1984. The increase of Ad3 strains relating to eye diseases occurred in 1986, and most agents were Ad3f. Ad3fT1H1, isolated in 1983, was identified again, and this subgenome type was dominant until August. Thereafter, this subgenome type was replaced with a new genome type, Ad3fH9T8. Ad3fH9T9, a possible derivative of Ad3fH9T8, was also isolated. Ad3fH9T8 seemed to be derived from Ad3fH1T1; only one of the fragments generated with the respective enzymes showed any difference. However, more fragments of Ad3fH2T2, Ad3fT4H5, and Ad3fH6T6 were different from those of Ad3fH1T1, suggesting that they were not derived from Ad3fH1T1. They might have been introduced into this locality from elsewhere.

An increase of Ad3 strains was observed in 1983. The major strain in 1983 was Ad3g, and a single subgenome type, Ad3fH1T1, was predominant. This subgenome type was also found in one strain in 1984. In 1985, Ad3gH2T1, a possible derivative of Ad3gH1T1, appeared and took the place of Ad3gH1T1, and all strains in 1986 were also of this subgenome type. One strain of a new subgenome type (Ad3gH3T2) was isolated in 1985.

The global distribution of adenovirus genome types is becoming well characterized through the efforts of molecular

TABLE 1. Subgenome types of chronologically arranged Ad3f and Ad3g strains

Time of isolation		Strain	Subgenome type
Yr	Mo/day		
1983	1/8	TC2944[a]	Ad3fH1T1
	4/15	TC3155[a]	Ad3fH2T2
	7/8	TC3360[a]	Ad3fH3T1
	7/22	TC3508	Ad3fH1T1
	3/30	TC3109[a]	Ad3gH1T1
	7/8	TC3378	Ad3gH1T1
	7/9	TC3380	Ad3gH1T1
	7/13	TC3383	Ad3gH1T1
	7/15	TC3391	Ad3gH1T1
	7/18	TC3409	Ad3gH1T1
	7/21	TC3399	Ad3gH1T1
	7/23	TC3424	Ad3gH1T1
	7/25	TC3426	Ad3gH1T1
	7/28	TC3433	Ad3gH1T1
	7/30	TC3447	Ad3gH1T1
	8/1	TC3469[a]	Ad3gH1T1
	8/10	TC3477	Ad3gH1T1
	8/22	TC3525	Ad3gH1T1
	10/24	TC3702[a]	Ad3gH1T1
	12/2	TC3793[a]	Ad3gH1T1
1984	7/13	V3934[a]	Ad3fH4T3
	8/10	TC4496[a]	Ad3fH5T4
	8/28	V4106[a]	Ad3fH4T5
	11/15	TC4813[a]	Ad3fH6T6
	7/25	V3941[a]	Ad3gH1T1
1985	8/19	V5143[a]	Ad3fH7T7
	8/30	V5188[a]	Ad3fH8T3
	9/17	V5230[a]	Ad3gH2T1
	12/13	V5473[a]	Ad3gH3T2
	12/13	TC6067[a]	Ad3gH2T1
1986	1/29	V5508[a]	Ad3fH1T1
	7/3	TC6791[a]	Ad3fH1T1
	7/12	TC6874[a]	Ad3fH1T1
	7/30	TC6966[a]	Ad3fH1T1
	8/18	TC7068[a]	Ad3fH1T1
	8/25	TC7080[a]	Ad3fH1T1
	8/26	TC7089[a]	Ad3fH1T1
	8/29	TC7150[a]	Ad3fH1T1
	9/13	TC7205[a]	Ad3fH9T8
	11/8	TC7542	Ad3fH9T8
	12/3	TC7649	Ad3fH1T1
	12/9	TC7678	Ad3fH9T9
	12/12	TC7680	Ad3fH9T8
	12/12	TC7681	Ad3fH9T8
	12/15	TC7682	Ad3fH9T8
	12/23	TC7713	Ad3fH1T1
	1/20	TC6148[a]	Ad3gH2T1
	1/22	V5505[a]	Ad3gH2T1
	3/24	TC6383[a]	Ad3gH2T1

[a] The genome types of these strains have been reported by Guo et al. (11).

epidemiologists. Li and Wadell (18–20) divided 15 Ad7 genome types, 17 Ad3 genome types, and 8 Ad4 genome types into three clusters and discussed the epidemic of adenovirus infections. They reported that Ad3a2 was predominant in genome type Ad3 in China from 1962 to 1985 and that Ad3a4, Ad3a5, and Ad3a6 have appeared along with Ad3a2 since 1983. O'Donnell et al. (21) identified six different Ad3 genome types (Ad3p and Ad3a to Ad3e) in 1981 in Glasgow, United Kingdom, and found that Ad3p, Ad3a, and Ad3c appeared repeatedly in 1982. Recently, Ad3 strains isolated from patients with acute conjunctivitis in Japan were classified into two genome types, Ad3f and Ad3g, with *Bam*HI, *Bgl*II, *Hin*dIII, and *Sma*I (11). In the present study, 49 Ad3 strains in Sapporo classified with the same REs were also found to be either Ad3f or Ad3g, and Ad3 strains isolated from 1983 to 1986 belonged to the simple genome types so far examined. In order to examine the epidemic of adenovirus infections in local microenvironments in detail, we used REs recognizing 4- or 5-base-pair sequences, and many subgenome types were identified among the genome types. Chronological arrangement of the subgenome types indicated the slight alteration of adenovirus DNA, even within the prevalent strains, and that some subgenome types seemed to derive from the subgenome types that had been identified while others were introduced to this area from elsewhere.

Increases of Ad3g in 1983 and Ad3f in 1986 were observed. All Ad3g strains isolated in 1983 belonged to a single subgenome type. However, in Ad3f, one possible derivative of the subgenome type that dominated until the middle of the increase became predominant late in the increase. In the years in which only a few Ad3f strains were isolated, subgenome types of such strains tended to be new members and their possible derivatives and were found only in those years. However, for Ad3g, one of these subgenome types was isolated in 1986 and became the predominant subgenome type. Adrian et al. (1) observed that some genome types were more likely to spread and to survive than others that were found only once and assumed differences in the replication cycle and viral release among genome types as one possible reason.

By additional digestion with *Sal*I (data not shown), we determined that genome types Ad3f and Ad3g reported by Guo et al. (11) would be Ad3a and Ad3c, respectively, according to the naming system proposed by Li and Wadell (19). To avoid confusion in adenovirus molecular epidemiology, it seems to be necessary to be standardized with regard to naming strains according to their restriction patterns.

By the method using REs recognizing 4- or 5-base-pair sequences, we could carry out the epidemiological analysis of strains Ad3f and Ad3g in detail. This method is a powerful tool for investigating the epidemiology of adenoviruses in local microenvironments.

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