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Intermediate Human Adenovirus Type 22/H10,19,37 as a New Etiological Agent of Conjunctivitis

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Eight strains of a new intermediate adenovirus were isolated in the course of an investigation of conjunctivitis in Hiroshima City, Japan. The strain was first isolated in July 1986. All eight strains were isolated from conjunctival swab samples from patients with conjunctivitis, mainly epidemic keratoconjunctivitis. The virus was typed as adenovirus type 22 in neutralization tests and was related closely to types 10, 19, and 37 in hemagglutination inhibition tests. The DNA cleavage patterns of the eight strains with nine restriction endonucleases were the same with one exception but different from those of the above serologically related species. We conclude that the intermediate adenovirus is a new etiological agent of conjunctivitis, mainly epidemic keratoconjunctivitis.

Human adenovirus is composed of 41 species plus six candidate species so far (6, 18) and divided into six different subgenera, A to F, based on their DNA homology (15). Among them, the 23 established species of subgenus D, which agglutinate rat erythrocytes as a common characteristic, are genetically closely related, and therefore some serological cross-reactions between different prototypes are observed (19). Furthermore, serologically intermediate-type isolates of adenovirus, which relate to one prototype in neutralization and to another in hemagglutination inhibition (HI), have been reported, mostly in subgenus D (17).

On the other hand, medically important species in subgenus D are adenovirus types 8, 19, and 37 (15). They mainly cause epidemic keratoconjunctivitis (EKC). In the course of a virological investigation of conjunctivitis begun in April 1983 in Hiroshima City, Japan (8), we have isolated eight strains of an uncommon adenovirus which differed from established adenoviruses as a causative agent of conjunctivitis. They were typed as adenovirus type 22 in neutralization tests and found to be closely related to types 10, 19, and 37 in HI tests.

In this article, we tentatively designate these viruses adenovirus type 22/H10,19,37 (Ad22/H10,19,37) and characterize them serologically and genetically.

MATERIALS AND METHODS

Clinical specimens and virus isolation. Conjunctival swab specimens for virus isolation were collected from 1,170 patients with various types of conjunctivitis at two ophthalmology clinics in Hiroshima City during the period from April 1983 to December 1989 (collection from one ended in April 1987). Virus isolation was done with HEp-2 cells, human embryonic fibroblast cells, and RD-18S cells as described previously (8). Isolated viruses, including Ad22/H10,19,37, were initially serotyped by neutralization tests with antisera provided by courtesy of the National Institute of Health, Tokyo, Japan, or with antisera from Ismunit Co., Rome, Italy.

Viruses. Prototypes of Ad1 through Ad41 except Ad36 and Ad38 were kindly supplied from the National Institute of Health, Tokyo. The prototype strains and adenovirus isolates were serially passaged several times in HEp-2 cells in our laboratory.

Antisera. Antisera to prototypes of Ad10, Ad19, Ad22, and Ad37 and to two isolates of Ad22/H10,19,37 (strains 87006C and 88249C) were prepared by repeated intramuscular injections into rabbits of a mixture of Freund's complete adjuvant and partially purified virions obtained from virus-infected HEp-2 cells by freeze-thawing, polyethylene glycol precipitation, and CsCl isopycnic centrifugation.

Antisera (20 to 100 U) to prototypes other than those mentioned above were the same as described under the heading "Clinical specimens and virus isolation."

HA and HI tests. Hemagglutination (HA) and HI tests were performed by a conventional method in U-type microtiter plates in 0.01 M phosphate-buffered saline at 37°C. An 0.5% suspension of erythrocytes from the various animals shown in Table 2 were used for the HA tests. The HI tests were performed with 0.5% rat erythrocytes. Antisera were treated at a final dilution of 1:10 with 25% kaolin and packed rat erythrocytes before use.

NT tests. Neutralization (NT) tests were performed on HEp-2 cells in 96-well microtiter plates. Challenge virus was used at a dilution that showed complete cytopathic effect (CPE) 4 days after the inoculation. Antisera diluted to 1:4 were inactivated at 56°C for 30 min and serially twofold diluted. The mixture of diluted serum and virus was incubated at 37°C for 2 h and then at 4°C overnight and inoculated onto the cells. The NT titer was expressed as the reciprocal of the highest dilution of serum that inhibited CPE completely 4 days after the inoculation. Delay in appearance of CPE was not considered neutralization.

DNA restriction enzyme analysis. DNA restriction enzyme analysis was carried out as described previously (12). Restriction endonucleases used in this study were *Bam*HI, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Kpn*I, *Pvu*II, *Sma*I, and *Xho*I, purchased from Toyobo Co., Osaka, Japan.

RESULTS

Isolation of Ad22/H10,19,37 from cases of conjunctivitis. Ad22/H10,19,37 had not been isolated until 1985. After the first isolation of the virus on 25 July 1986, a total of eight Ad22/H10,19,37 strains were isolated by the end of 1988 (Table 1). All Ad22/H10,19,37 strains were isolated from

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TABLE 1. Source of Ad22/H10,19,37 isolates

Strain no.	Clinical diagnosis[a]	Sex of patient	Age (yr)	Sampling date (days after onset)
86316C	EKC	M	35	25 July 1986 (3)
86355C	EKC	M	36	4 Aug 1986 (3)
87003C	AHC	M	23	5 Jan 1987 (5)
87006C	EKC	F	20	6 Jan 1987 (3)
87040C	AC	F	32	29 Jan 1987 (3)
87050C	EKC	M	23	31 Jan 1987 (7)
88249C	EKC	M	47	22 June 1988 (2)
88501C	EKC	F	23	14 Nov 1988 (5)

[a] Abbreviations: AHC, acute hemorrhagic conjunctivitis; AC, acute conjunctivitis.

conjunctival swab specimens taken from eight patients aged 20 to 47 years in the acute phase of conjunctivitis. Of the eight cases, six were clinically diagnosed as EKC, one as acute hemorrhagic conjunctivitis, and the other as acute conjunctivitis. No other virus was isolated from these samples. No information about the epidemiological relationships among the patients was obtained.

All eight strains were easily isolated in both HEp-2 cells and human embryo fibroblast cells, and four of them were also isolated in RD-18S cells. Ad22/H10,19,37 showed typical round-type CPE in the HEp-2 cells.

HA activity of Ad22/H10,19,37. Table 2 shows the HA activities on various animal erythrocytes of Ad22/H10,19,37 and the reference strains. Ad22/H10,19,37 completely agglutinated rat erythrocytes, indicating that this virus is in subgenus D. The virus also agglutinated human O, guinea pig, and mouse erythrocytes at almost the same titers as rat erythrocytes and green and cynomolgus monkey erythrocytes at significantly lower titers.

Among the strains tested, the HA pattern of Ad22/H10,19,37 was the same as that of Ad19 strains isolated in Hiroshima City (Ad19a) (12).

Identification of Ad22/H10,19,37 by NT tests. All Ad22/H10,19,37 strains were neutralized by anti-Ad22 prototype serum; conversely, Ad22 prototype was neutralized by both anti-87006C and anti-88249C sera, indicating that the strains are serotyped as Ad22 by NT tests (Table 3). Weak cross-reactions (delay of CPE) were observed among Ad22/H10,19,37, Ad10, Ad19, and Ad37.

No prototype of Ad1 through Ad41 (except Ad36 and Ad38, which were not available at our laboratory) was neutralized by the anti-88249C serum, and two strains of Ad22/H10,19,37 (87006C and 88249C) were not neutralized by antisera against prototypes of Ad1 through Ad9, Ad11 through Ad18, Ad20, Ad21, Ad23, Ad24, Ad28, Ad30, and Ad31 through Ad35.

TABLE 3. Identification of Ad22/H10,19,37 by cross-neutralization tests

Virus	NT titer[a]					
	Ad10	Ad19	Ad37	Ad22	87006C	88249C
Ad10 prototype	**16**	—[b]	—	—	—	—
Ad19 prototype	—	**512**	—	—	—	—
Ad37 prototype	—	—	**256**	—	—	—
Ad22 prototype	—	—	—	**256**	128	256
Ad22/H10,19,37 isolates						
87006C	—	—	—	512	**256**	512
88249C	—	—	—	512	256	**512**
Others (n = 6)	—	—	—	512–1,024	128–512	256–1,024

[a] Values in boldface are homologous titers.
[b] —, <4.

Identification of Ad22/H10,19,37 by HI tests. All Ad22/H10,19,37 strains reacted to the antisera against Ad10, Ad19, and Ad37 in the same way as to each homologous virus and did not react to the anti-Ad22 prototype serum (Table 4). Conversely, Ad10, Ad19, and Ad37 reacted to the anti-Ad22/H10,19,37 serum.

None of the 19 kinds of prototype strains in subgenus D, except Ad36, Ad38 and also Ad25 and Ad28, which did not agglutinate rat erythrocytes under the condition used, reacted to the anti-88249C serum.

DNA cleavage patterns of Ad22/H10,19,37. The DNA cleavage patterns of Ad22/H10,19,37 and the serologically related viruses are shown in Fig. 1. All of the Ad22/H10,19,37 strains except 88249C showed identical patterns with the nine restriction endonucleases used. The 88249C strain showed a different pattern from the other Ad22/H10,19,37 strains with *Pvu*II and the same patterns with the other eight enzymes. On the other hand, the patterns of Ad22/H10,19,37 strains differed from those of prototypes of Ad22, Ad10, Ad19, Ad37, and Ad19a. However, similarities in cleavage patterns among them were observed, showing that Ad22/H10,19,37 is genetically classified in subgenus D.

To ensure the genetic relatedness between Ad22/H10,19,37 and Ad22, Ad10, Ad19, or Ad37, comigration analysis of the digested DNAs was performed (Table 5). The percentages of comigrating fragments between Ad22/H10,19,37 (strain 87006C) and each of the prototypes of Ad22, Ad10, Ad19, Ad37, and Ad19a (strain 86143C) were 59, 63, 52, 66, and 65%, respectively.

TABLE 2. HA titers of Ad22/H10,19,37 and serologically related viruses

Virus (no. of strains)	HA titer on erythrocytes from[a]:							
	Rat	Human O	Mouse	GP	GM	CM	Sheep	Goose
Ad22/H10,19,37 isolate[b] (8)	128	256–512	256–512	64–256	1–2	1–2	—[c]	—
Ad10 prototype	64	256	512	—	—	—	—	—
Ad19 prototype	64	256	512	—	1	—	—	—
Ad37 prototype	128	256	256	128	16	8	—	—
Ad22 prototype	512	—	1,024	—	—	—	—	—
Ad19 isolates[b] (4)	128–256	256	256–512	64–128	1–2	1–2	—	—
Ad37 isolates[b] (6)	64–128	256	256–512	64–128	8–32	8–32	—	—

[a] Abbreviations: GP, guinea pig; GM, green monkey; CM, cynomolgus monkey.
[b] Strains isolated in Hiroshima City.
[c] —, <1.

TABLE 4. Identification of Ad22/H10,19,37 by cross-HI tests

Virus	HI antibody titer[a]					
	Ad10	Ad19	Ad37	Ad22	87006C	88249C
Ad10 prototype	**80**	320	160	—[b]	160	640
Ad19 prototype	40	**640**	320	—	160	640
Ad37 prototype	80	1,280	**1,280**	—	320	2,560
Ad22 prototype	—	—	—	**2,560**	—	—
Ad22/H10,19,37 isolates						
87006C	80	1,280	1,280	—	**640**	2,560
88249C	80	1,280	1,280	—	640	**2,560**
Others (n = 6)	80	1,280	1,280–2,560	—	640	1,280–2,560

[a] Values in boldface are homologous titers.
[b] —, <10.

DISCUSSION

The results of the NT and HI tests showed that our adenovirus isolates are intermediate-type viruses related to Ad22 in the NT tests and to Ad10, Ad19, and Ad37 in the HI tests. So far, some intermediate adenovirus isolates (hemagglutinin variants) have been reported in subgenus D (17). No virus isolate possessing the same antigenicity as our strains has been reported, indicating that our type is a new one. The results of DNA restriction enzyme analysis also showed that all eight strains of Ad22/H10,19,37 are genetically identical but different from the above-mentioned serologically related viruses. In this study, we could not examine relationships between Ad22/H10,19,37 and some species, especially Ad36 and Ad38. No significant cross-reactivity in the NT and HI tests among Ad36, Ad38, Ad22, Ad10, Ad19, and Ad37 has been observed in other studies (7, 19). Weak cross-reactions in the NT tests among Ad22/H10,19,37, Ad10, Ad19, and Ad37 may be due to the strong cross-reactivities among them in the HI tests, as described by Wigand et al. (19).

All Ad22/H10,19,37 strains were isolated from conjunctival swab specimens from eight patients in the acute phase of conjunctivitis. Six of them were from patients diagnosed clinically as having EKC. No other virus was isolated from the same samples. Taking these facts together, we conclude that Ad22/H10,19,37 is a new etiological agent of conjunctivitis, mainly EKC, although we did not confirm the infection of the patients with it serologically.

It is interesting to speculate on the origin of Ad22/H10,19,37 strains from the viewpoint of the evolution of adenoviruses. Intermediate types of adenoviruses are generally thought to be created by the recombination of two different species (1, 14). This idea suggests that Ad22/H10,19,37 is a recombinant of Ad22, with the neutralization-related ε antigen located outside the hexon (13) and Ad10, Ad19, or Ad37, with the hemagglutinin, or γ antigen, located at the top of the fiber (13). Ad19a seems to be the most plausible candidate from the similarities in the HA pattern (Table 2), although the results of the HI tests and comigration analysis of the cleaved DNAs cannot conclusively determine whether Ad10, Ad19, or Ad37 is the donor of the hemagglutinin. This hypothesis is supported by the epidemi-

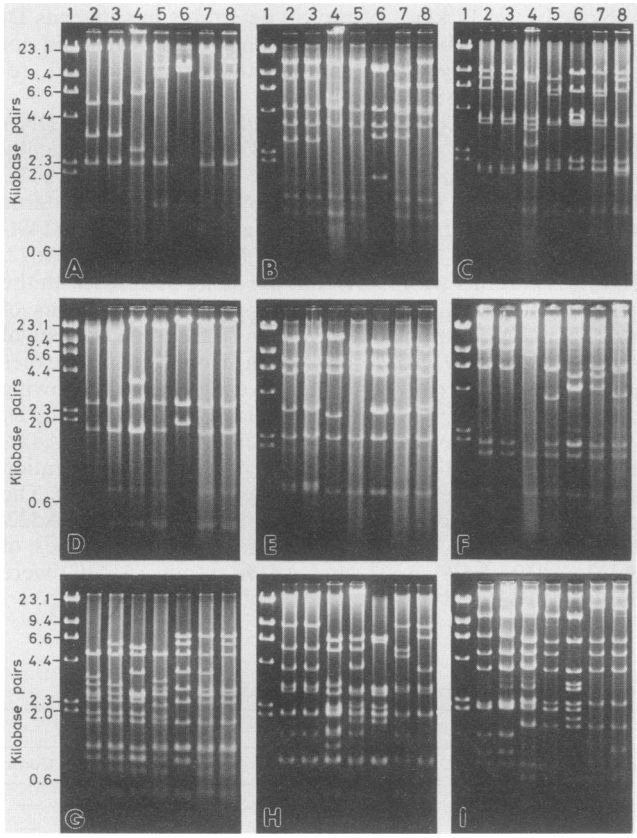

FIG. 1. DNA cleavage patterns of Ad22/H10,19,37 and serologically related viruses, obtained after digestion with restriction endonucleases *Bam*HI (A), *Bgl*II (B), *Bst*EII (C), *Eco*RI (D), *Hin*dIII (E), *Kpn*I (F), *Pvu*II (G), *Sma*I (H), and *Xho*I (I). Lanes 1, reference markers (*Hin*dIII digest of lambda DNA). Lanes 2, Ad22/H10,19,37 (strain 87006C). Lanes 3, Ad22/H10,19,37 (strain 88249C). Lanes 4, Ad22 prototype. Lanes 5, Ad10 prototype. Lanes 6, Ad19 prototype. Lanes 7, Ad19a (strain 86143C). Lanes 8, Ad37 prototype.

TABLE 5. Comigration analysis comparison of Ad22/H10,19,37 and serologically related viruses

Virus strain	% Comigrating fragments[a]						
	87006C (78)[b]	88249C (77)	Ad22 (81)	Ad10 (78)	Ad19 (75)	Ad19a (74)	Ad37 (73)
87006C	100	98	59	63	52	65	66
88249C	98	100	58	63	54	65	68

[a] Calculated as follows: [number of comigrating fragments between Ad22 and 87006C/(total number of fragments of Ad22 + total number of fragments of 87006C)] × 100.
[b] Numbers in parentheses indicate the total number of fragments obtained after cleavage with the nine restriction endonucleases described in the legend to Fig. 1.

ological fact that Ad19a has been isolated frequently all over the world, including Hiroshima City (8–10).

Why Ad22/H10,19,37 suddenly began to be isolated from patients with conjunctivitis in Hiroshima City is not clear. One possible explanation is as follows. Ad22 was first isolated from a patient with trachoma in Saudi Arabia in 1956 (2). Since then, Ad22 has been isolated rarely (6, 9, 20, 21). According to World Health Organization reports (20, 21), only three isolates of Ad22, two from gastroenteritis and one from cardiovascular disease, were reported worldwide from 1981 to 1983. In a virological investigation of diseases, including eye disease (conjunctivitis), in Australia from 1972 to 1979 by Irving et al. (9), no strain of Ad22 was isolated from eye specimens, though nine strains of Ad22 were isolated from other specimens. These reports suggest that the affinity of Ad22 for the conjunctiva was originally weak, with or without the ability to cause conjunctivitis. It is reasonable to speculate that Ad22 increased its ability to infect the conjunctiva by getting the fiber, which is thought to work on the first step of infection (3), from other species of adenoviruses, with or without a change in some other region of the genome that effects pathogenic processes (5). Ad10, Ad19, and Ad37 have all been shown to be causative agents of conjunctivitis (4, 10, 11, 16). The idea that Ad22/H10,19,37 came from the recombination of Ad22 and Ad10, Ad19, or Ad37 may explain the mechanism of the sudden appearance of Ad19a and Ad37 as causative agents of conjunctivitis, as reported previously (4, 10).

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REFERENCES

1. **Adrian, T., B. Bastian, and V. Wagner.** 1989. Restriction site mapping of adenovirus types 9 and 15 and genome types of intermediate adenovirus 15/H9. Intervirology **30**:169–176.
2. **Bell, S. D., T. R. Rota, and D. E. McComb.** 1960. Adenoviruses isolated from Saudi Arabia. III. Six new serotypes. Am. J. Trop. Med. Hyg. **9**:523–526.
3. **Brown, D. T., and B. T. Burlingham.** 1973. Penetration of host cell membranes by adenovirus 2. J. Virol. **12**:386–396.
4. **de Jong, J. C., R. Wigand, G. Wadell, D. Keller, C. J. Muzerie, A. G. Wermenbol, and G. J. P. Schaap.** 1981. Adenovirus 37: identification and characterization of a medically important new adenovirus type of subgroup D. J. Med. Virol. **7**:105–118.
5. **Ginsberg, H. S., U. Lundholm-Beauchamp, R. L. Horswood, B. Pernis, W. S. M. Wold, R. M. Chanock, and G. A. Prince.** 1989. Role of early region 3 (E3) in pathogenesis of adenovirus disease. Proc. Natl. Acad. Sci. USA **86**:3823–3827.
6. **Hierholzer, J. C., R. Wigand, L. J. Anderson, T. Adrian, and J. W. M. Gold.** 1988. Adenoviruses from patients with AIDS: a plethora of serotypes and a description of five new serotypes of subgenus D (types 43–47). J. Infect. Dis. **158**:804–813.
7. **Hierholzer, J. C., R. Wigand, and J. C. de Jong.** 1988. Evaluation of human adenoviruses 38, 39, 40, and 41 as new serotypes. Intervirology **29**:1–10.
8. **Ikeda, Y., M. Noda, Y. Otagaki, T. Matsuishi, and T. Ogino.** 1988. Virus isolation from acute conjunctivitis in Hiroshima City. Rinsho To Uirusu [Clin. Virol.] **16**:348–353. (In Japanese.)
9. **Irving, L., M. Kennett, F. Lewis, C. Birch, and A. Donaldson.** 1981. Adenovirus eye infections in an Australian city, 1972–9. J. Hyg. (Cambridge) **86**:95–103.
10. **Kemp, M. C., J. C. Hierholzer, C. P. Cabradilla, and J. F. Obijeski.** 1983. The changing etiology of epidemic keratoconjunctivitis: antigenic and restriction enzyme analyses of adenovirus types 19 and 37 isolated over a 10-year period. J. Infect. Dis. **148**:24–33.
11. **Newland, J. C., and M. K. Cooney.** 1978. Characteristics of an adenovirus type 19 conjunctivitis isolate and evidence for a subgroup associated with epidemic conjunctivitis. Infect. Immun. **21**:303–309.
12. **Noda, M., Y. Otagaki, Y. Ikeda, T. Matsuishi, and T. Ogino.** 1988. Genome types of adenovirus types 19 and 37 isolated from patients with conjunctivitis in Hiroshima City. J. Med. Virol. **26**:15–22.
13. **Norrby, E.** 1969. The structural and functional diversity of adenovirus capsid components. J. Gen. Virol. **5**:221–236.
14. **Sambrook, J., M. Sleigh, J. A. Engler, and T. R. Broker.** 1980. The evolution of the adenoviral genome. Ann. N.Y. Acad. Sci. **354**:426–452.
15. **Wadell, G.** 1984. Molecular epidemiology of human adenoviruses. Curr. Top. Microbiol. Immunol. **110**:191–220.
16. **Wadell, G., and J. C. de Jong.** 1980. Restriction endonucleases in identification of a genome type of adenovirus 19 associated with keratoconjunctivitis. Infect. Immun. **27**:292–296.
17. **Wigand, R., and T. Adrian.** 1989. Intermediate adenovirus strains of subgenus D occur in extensive variety. Med. Microbiol. Immunol. **178**:37–44.
18. **Wigand, R., T. Adrian, and F. Bricout.** 1987. A new human adenovirus of subgenus D: candidate adenovirus type 42. Arch. Virol. **94**:283–286.
19. **Wigand, R., D. Keller, and I. Werling.** 1982. Immunological relationship among human adenoviruses of subgenus D. Arch. Virol. **72**:199–209.
20. **World Health Organization.** 1985. WHO virus report 1981. Rinsyo To Uirusu [Clin. Virol.] **13**:186–198. (In Japanese; translated by K. Yamashita, The [Japanese] National Institute of Health.)
21. **World Health Organization.** 1988. WHO virus report 1982–1983. Rinsyo To Uirusu [Clin. Virol.] **16**:97–116. (In Japanese; translated by K. Yamashita, The [Japanese] National Institute of Health.)