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Further characterization of 41 isolates of adenovirus types 19/37 by serum neutralization and DNA restriction enzyme analysis

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SUMMARY

Forty-one strains of adenovirus type 19/37 (Ad19/37) mainly isolated from patients with keratoconjunctivitis or conjunctivitis between 1974 and 1984 were re-evaluated by serum neutralization (SN), haemagglutination inhibition (HI) and DNA restriction analysis. Of 19 isolates which were neutralized to high titre by antiserum prepared against prototype Ad19, 5 showed cross-reactivity with 32–64 units of Ad37 antiserum, while of 22 strains neutralized to high titre by Ad37 antiserum, 3 showed cross-reactivity with 32 units of Ad19 antiserum. By DNA restriction analysis, all Ad19 isolates were identical to each other and to Ad19A virus. Using endonuclease Bgl 1, three variants were observed among the Ad37 isolates.

INTRODUCTION

In the last decade adenovirus type 19 (Ad19) (Desmyter *et al.* 1974) and 37 (Ad37) (Schaap *et al.* 1979; Australian Department of Health, 1981; de Jong *et al.* 1981; Keenlyside, Hierholzer & D'Angelo, 1983; Hammond *et al.* 1985) have been recognized as two of the main causes of epidemic keratoconjunctivitis (EKC). Studies in recent years have demonstrated that between one-third and two-thirds of strains of adenovirus initially classified as Ad19 by haemagglutination inhibition were actually Ad37 (Kemp *et al.* 1983; Aoki *et al.* 1985) because a clear relationship between Ad37 and Ad19 has been found in both directions (de Jong *et al.* 1981).

During 1981–4, a number of adenovirus strains which appeared to be neutralized by both Ad19 and Ad37 antisera were isolated at Fairfield Hospital. This study was designed to clarify the identity of these and earlier isolates, and to determine whether genetic variants of Ad19 and Ad37 types exist.

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MATERIALS AND METHODS

Origin of adenovirus strains

The prototype of adenovirus type 19 (3911) (Ad19P) was received from the Center for Disease Control, Atlanta, Georgia, USA, and that of adenovirus type 37 (76-19026) (Ad37P) from Dr de Jong, Rijksinstituut, The Netherlands. Strain 128048 was isolated from an eye swab from a patient with EKC in Melbourne in 1974 and identified as Ad19A by Dr de Jong. Strain 205452 was isolated from a urethral swab in 1984, identified as Ad37 by the serum neutralization test (SN) and denoted Ad37U. Forty strains of Ad19 or 'Ad19/37' which exhibited cross-reactivity to Ad19 and 37 antisera in preliminary identification were isolated from corneal or conjunctival swabs or scrapings from patients with keratoconjunctivitis or conjunctivitis at the Virology Department, Fairfield Hospital between 1974 and 1984.

Type-specific antisera

In this study, five type-specific antisera were used. Anti-Ad19P serum was obtained from the National Institutes of Health, USA (research reference catalogue N219501-561), anti-Ad37P serum was received from Dr de Jong, and anti-Ad19A, anti-Ad37P and anti-Ad37U sera were raised in rabbits at Fairfield Hospital by standard procedures (de Jong *et al.* 1981).

Serum neutralization (SN) tests

SN tests were performed as described previously (Irving & Smith, 1981). Fourfold dilutions of antisera were tested against 30–300 TCD_{50} of each virus isolate.

Haemagglutination (HA) and haemagglutination inhibition (HI) tests

HA and HI tests were similar to those described previously (de Jong *et al.* 1981) except that 0·5 % (v/v) human, mouse, rat, guinea-pig and dog erythrocytes were used.

Restriction enzyme analysis of viral DNA

The extraction of viral DNA was carried out by a rapid and simple method (Shinagawa *et al.* 1983), except that all centrifugation steps were performed in an Eppendorf microfuge (10000 rev./min for 3 min to remove cellular DNA, 10000 rev./min for 10 min to pellet viral DNA) and proteinase K was used instead of protease type V1. For restriction enzyme analysis, 1 μl of restriction enzyme (Sma 1 or Bgl 1) was added to 20 μl of DNA solution in a final volume of 30 μl appropriate digestion buffer and incubated at 30 °C (Sma 1) or 37 °C (Bgl 1) overnight. After addition of electrophoresis sample-loading buffer containing bromophenol blue, the mixtures were electrophoresed through 0·7 % agarose in tris-acetate buffer (pH 7·5) for 4 h at 40 V in the presence of ethidium bromide. The resultant electrophoretic profiles were recorded by direct photography of u.v.-illuminated slab gels.

Table 1. *Cross-SN test between Ad19 and Ad37 viruses*

Type	Strain	Number	SN titres of antisera			
			Ad19P	Ad19A	Ad37P	Ad37U
Ad19P	3911	1	3200	12800	< 200	< 200
Ad19A	128048	1	3200	6400	< 200	200
Ad19*		19	400–12800	400–12800	200 (4) 400 (1)	200 (4)
Ad37P	76-19026	1	< 200	< 200	12800	3200
Ad37U	205452	1	< 200	< 200	12800	12800
Ad37*		21	< 200	200 (3)	1600–12800	400–12800

Figures in parentheses are the number of reacted strains.
* Isolates from eye swabs or scrapings.

Table 2. *Haemagglutinin titres to adeno 19 and 37 isolates*

Virus (Number of strains tested)		Source of erythrocytes				
		Human	Rat	Mouse	Guinea-pig	Dog
Adeno 19 prototype		8	32	64	2	2
Adeno 37 prototype		8	32	16	< 2	< 2
Adeno 19 isolates (18)	Range	16–512	8–1024	8–512	2–64	< 2
	Median	64	64	32	32	
	GMT	81	64	49	17	
Adeno 37 isolates (19)	Range	2–512	< 2–512	< 2–1024	< 2–64	< 2
	Median	128	128	32	64	
	GMT	103	89	38	28	

The haemagglutinin (HA) titre was defined as the reciprocal of the highest dilution causing complete HA with 0·5% erythrocyte suspensions in basal medium (Eagle's) containing 0·05% bovine albumin fraction V in 1 h at 25 °C.
GMT, geometric mean titres.

RESULTS

Serum neutralization (Table 1)

The antisera prepared against Ad19P, 19A, 37P and 37U showed high homologous titres with no cross-reactivity between Ad19 and Ad37. Of 41 isolates tested, 19 were neutralized by dilutions of 1/4000–1/12800 of both Ad19A and 19P antisera; 5 of these viruses were also neutralized by 1/200–1/400 dilutions of antisera to Ad37P or Ad37U. Twenty-two strains were neutralized to high titre by anti-Ad37P and 37U sera; 3 of these viruses also reacted with Ad19A antiserum diluted to 1/200, but not with Ad19P antiserum.

Haemagglutinin titrations (Table 2)

No difference in mean haemagglutinin titres could be observed for 18 strains of Ad19 and 19 strains of Ad37 isolates using human, rat, mouse, guinea-pig or dog erythrocytes.

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Table 3. *Cross-HI test between Ad19 and Ad37 viruses*

Type	Strain	HI titre of antisera			
		Ad19P	Ad19A	Ad37P	Ad37U
Ad19P	3911	640	640	640	160
Ad19A	128048	640	640	640	640
Ad19	177476	160	2560	2560	640
Ad19	190375	640	640	640	640
Ad19	195760	640	2560	2560	2560
Ad19	210203	1280	1280	1280	1280
Ad37P	76-19026	160	640	2560	2560
Ad37U	205452	320	1280	1280	1280
Ad37	176500	320	1280	5120	1280
Ad37	195644	320	1280	5120	1280

Haemagglutination inhibition (Table 3)

By HI with human erythrocytes, a clear serological relationship between Ad19 and Ad37 was observed. Haemagglutination of strains identified by SN to be Ad19 could be inhibited to the same titre by both Ad19 and Ad37 antisera. Haemagglutination of strains identified by SN to be Ad37, however, could be inhibited to a higher titre by Ad37 antisera than by Ad19 antisera.

Restriction enzyme analysis of Ad19 and 37 viral DNA (Fig.1)

Two endonucleases (Sma 1 and Bgl 1) were used to examine 41 isolates which had been identified as Ad19 or 37 by SN. Twenty isolates of Ad19, including Ad19A (128048), showed an identical restriction pattern, indicating an absence of genetic variation in Ad19A isolates. The pattern was markedly different to that exhibited by Ad19P.

Of 22 isolates of Ad37, including Ad37U (205452), 21 strains showed the same Sma 1 restriction pattern as Ad37P, and one strain (192946) showed a different pattern (Fig. 1a, profile D). The second highest molecular weight band of DNA of this strain migrated more rapidly than its counterpart from Ad37P, and the third highest molecular weight band, in contrast, migrated more rapidly than its counterpart from strain Ad19A. This strain was isolated from an eye swab in 1983, and was one of three strains that exhibited cross-reactivity to Ad19A antiserum by SN. Using Bgl 1 restriction endonuclease, three distinct patterns were exhibited by strains 192946, 202452 (Ad37U), and the prototype Ad37P (Fig. 1b). Thus three distinct genomic variants of Ad37 were observed in this study.

DISCUSSION

The prototype Ad19 was isolated in 1955, and its role in EKC demonstrated in Denmark in 1973 (Desmyter *et al.* 1974). Subsequently, it has been found to cause disease in many countries (Hierholzer *et al.* 1974; Bell & Winton, 1975; Guyer *et al.* 1975; Wadell & de Jong, 1980; Irving *et al.* 1981). The Ad19 strains isolated from EKC patients could not be distinguished from Ad19 prototype strain by serological methods, although Wadell and colleagues have demonstrated that the

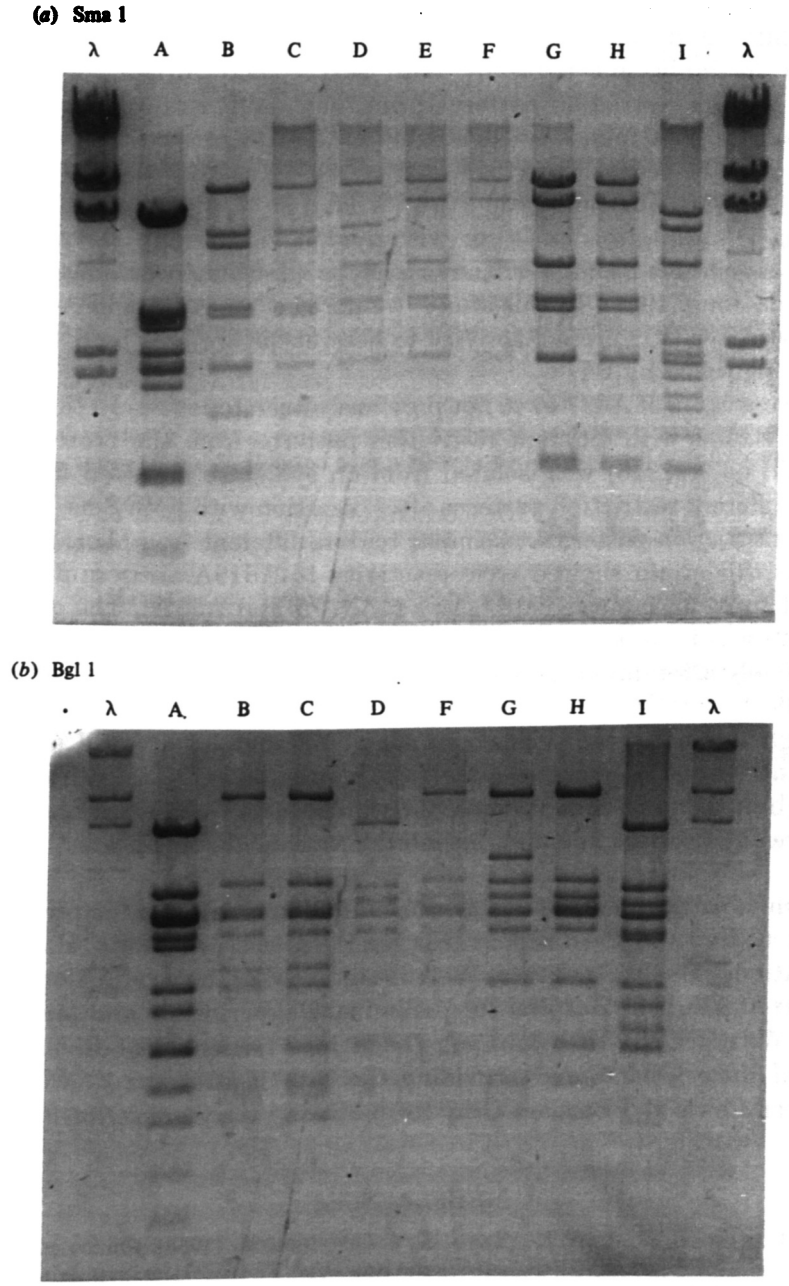

Fig. 1. Restriction enzyme patterns of adenoviruses. A, Ad19P (3911); B (strain 128048-1974) and C (strain 210103-1984), Ad19A; H, Ad37P(76-19026; D (strain 192946-1983), E (strain 195644-1983) and F (strain 207325-1984), Ad37 isolates, (D was a genomic variant); G (strain 205452-1984, from urethra), Ad37, a variant; I, Ad8. λ, Lambda DNA cleaved with HindIII.

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genetic type of EKC strains was different from Ad19P. Our study confirms their results. While 19 strains of Ad19 isolates from patients with EKC could be neutralized to equivalent titres by both Ad19P and Ad19A antisera, they exhibited different restriction patterns from the Ad19P strain. By contrast, no genomic variant was found in 19 isolates of Ad19A, even though they were isolated over an 11-year period (1974–84).

Ad37 was isolated in Europe in 1976 (Schaap *et al.* 1979). It was originally believed to be an intermediate type within adenovirus group D, but was subsequently classified as a new adenovirus type (de Jong *et al.* 1981; Wadell, Sundell & de Jong, 1981). In this study, we noted some Ad19 and Ad37 isolates which showed low-titre cross-reactivity to heterotype antisera by SN, but could not be distinguished by HI.

Genomic variants of Ad37 have not previously been reported. In this study, we found two strains with different restriction patterns from the prototype Ad37. One, (strain no. 192946) was isolated from an eye swab collected in 1983, and exhibited different restriction patterns after digestion with both Sma 1 and Bgl 1. Its Sma 1 restriction pattern was similar to, but different from, both Ad37P and Ad19A. As this strain showed cross-reactivity to Ad19A antiserum by SN, we consider it to be an intermediate between Ad19A and Ad37P. The other (strain no. 205452) was isolated from a urethral swab and showed differences from the prototype only after digestion with Bgl 1; no difference was seen after treatment with Sma 1.

Although the more traditional methods of SN and HI are still preferred for initial identification of adenovirus isolates, restriction endonuclease analysis has proved to be most useful in confirming these findings, and has potential applications in the investigation of epidemics, including nosocomial spread.

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