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Persistent enteral infections with adenovirus types 1 and 2 in infants: no evidence of reinfection

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SUMMARY

Isolates of adenovirus types 1 and 2, obtained from 11 infants with prolonged faecal excretion (up to 515 days), were compared by DNA restriction analysis with seven standard endonucleases which recognize hexanucleotides and two additional endonucleases which recognize tetranucleotides. In all instances identical genome types were identified in isolates obtained early and late after infection. Our interpretation of these data is that a chronic persistent infection occurred in these children, and not a reinfection with the same serotype.

During studies on the prevalence and spread of viruses in urban communities, undertaken as Virus Watch Programs in various parts of the United States (Fox *et al.* 1969; Monto, Napier & Metzner, 1971; Fox, Hall & Cooney, 1977), a prolonged faecal excretion of adenovirus (AV) types 1, 2 and 5 was observed in a number of infants. Either permanent or intermittent shedding was observed lasting 1 year or even longer (Brandt, Wasserman & Fox, 1966; Brandt *et al.* 1972). This phenomenon could be due either to a chronic persistent infection of the intestine or by reinfection with the same serotype, since AV1, 2 and 5 circulate extensively in small children (Brandt *et al.* 1972; Schmitz, Wigand & Heinrich, 1983; Pacini, Collier & Henderson, 1987). To decide between these two possibilities, viruses isolated early and late after infection were compared by DNA restriction analysis. In this context it is advantageous that adenoviruses of subgenus C (types 1, 2, 5, 6) display a high degree of genetic variability (Adrian, Best & Wigand, 1985; Wigand & Adrian, 1986).

Viruses were isolated from stool specimens in Seattle in human embryonic kidney (HEK) cell cultures and identified by neutralization. For DNA analysis, viruses were propagated in HeLa cells at Homburg. About 2×10^6 infected cells yielded sufficient DNA.

Table 1. *Enzyme code of adenovirus genome types*

AV 1 genome types									
D4	2	2	2	1	3	1	1	2	1*
D7	2	1	2	1	2	1	1	3	1
D10a	2	1	3	1	4	1	1	2	1
D10b	2	1	3	1	4	1	1	3	1
AV 2 genome types									
D2a	1	1	2	1	1	1	1	1	1
D2b	1	1	2	1	2	1	1	2	1
D12	1	1	2	2	3	2	1	3	2
	BamH I	Bgl II	BstE II	EcoR I	Hae III	Hind III	Kpn I	Msp I	Sma I

* DNA restriction patterns corresponding to those shown in Figures 1 to 3 (Hae III not shown). (1) is the DNA pattern of the respective prototype, (2) etc. those of the restriction variants.

Table 2. *Adenovirus isolates and genome types*

Virus type	Case number	Age (months)	Years of isolation	Days after first specimen	Genome type
1	1	16	66/67	0, 117, 155, 470	D7
	2	9	66/67	27, 275	D4
	3	18	66/68	18, 490	D10a
	4	2	67/68	0, 30, 69	D10b
	5	17	67/68	0, 170, 300, 515	D4
	6	13	68/69	0, 380	D4
2	7	11	66/67	0, 335	D2b
	8	15	66/67	0, 453	D2a
	9	14	66/67	0, 70, 200, 420	D12
	10	15	67/68	0, 250, 430	D2a
	11	14	68/69	15, 420	D2a

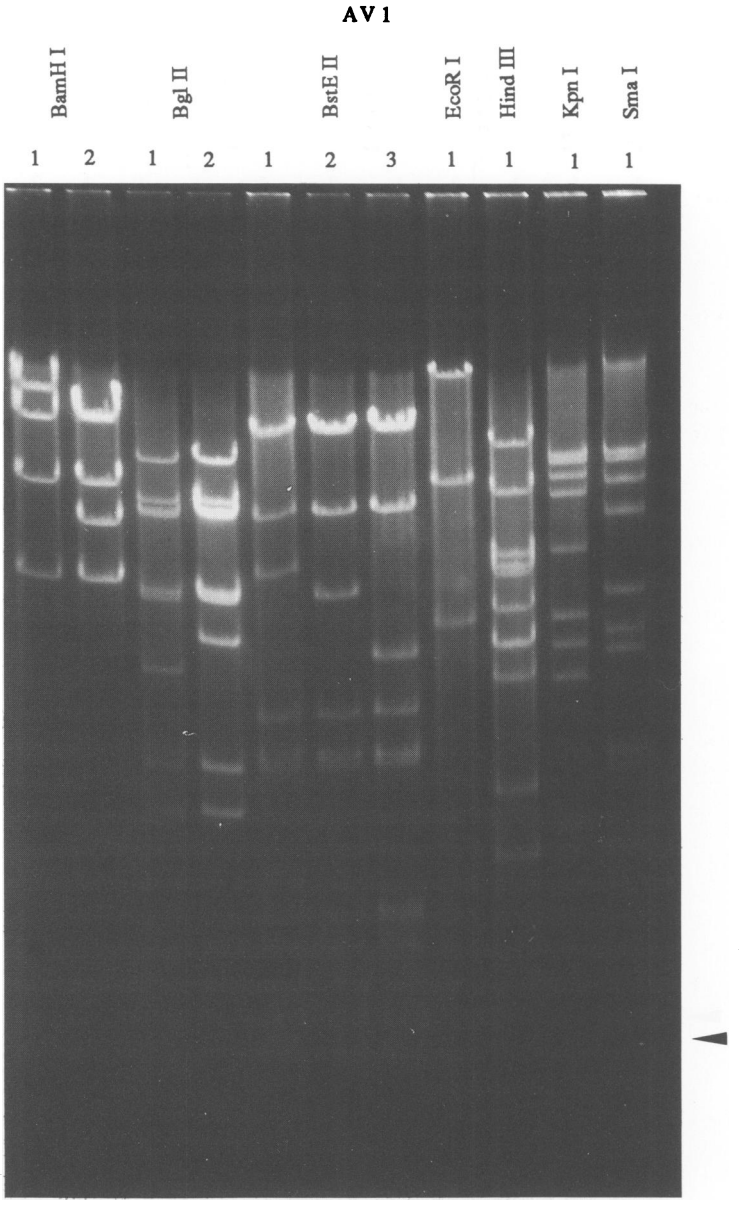

Fig. 1. DNA restriction patterns of AV 1 prototype (lane 1) and deviating restriction patterns (see Table 1) with the indicated seven enzymes. The arrow points to two small faint BstE II fragments.

DNA was extracted from infected HeLa cells (Wadell *et al.* 1980) and digested with nine restriction enzymes (BamH I, Bgl II, BstE II, EcoR I, Hae III, Hind III, Kpn I, Msp I and Sma I) from Boehringer, Mannheim, FRG. They were applied according to the published protocols of the company. DNA fragments were separated in 1·0 and 1·2% agarose gels.

We used the nomenclature proposed earlier (Adrian, Best & Wigand, 1985, see Table 1). D1 etc. denotes the genome types ('D' for DNA). Seven restriction enzymes were applied to all strains and listed in alphabetical order.

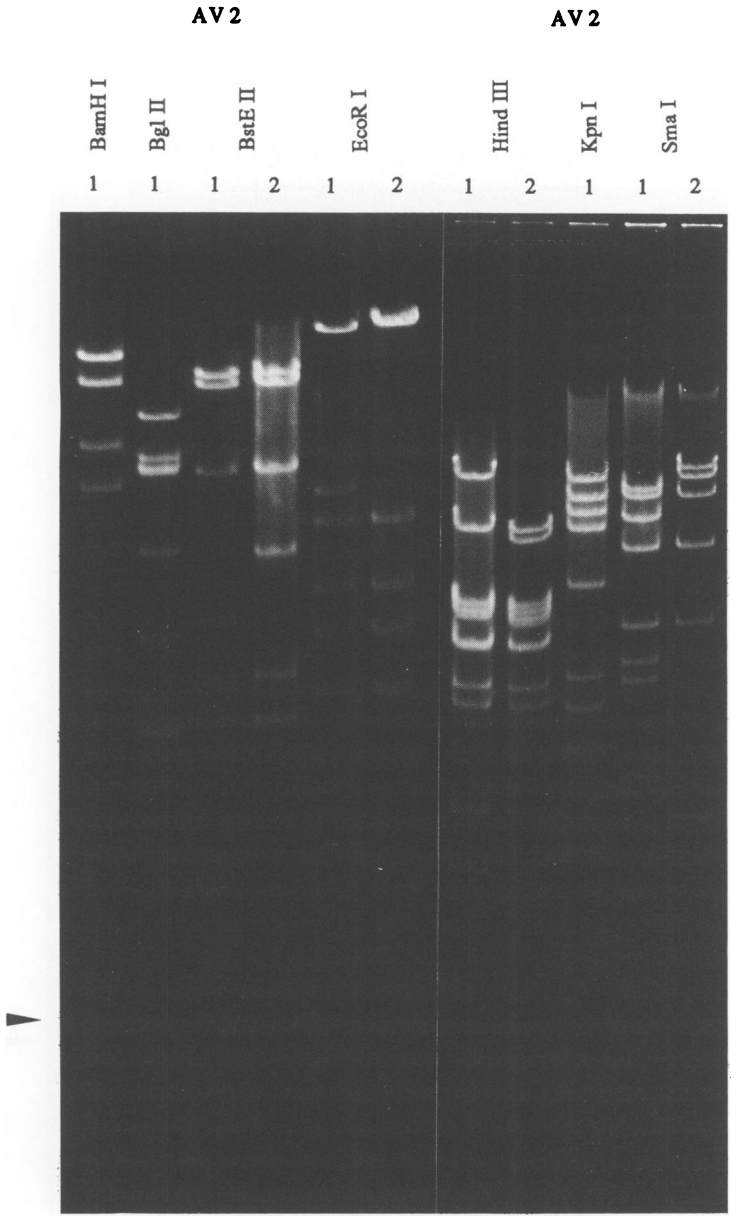

Fig. 2. DNA restriction patterns of AV 2 prototype (lane 1) and deviating restriction patterns (see Table 1) with seven enzymes. The arrow points to two small faint BstE II fragments.

The figures indicate restriction patterns; those noted as (1) correspond to the prototype (D1). D2, D3 etc. are genome types differing in one or more enzymes from the prototype. To ensure identity of the genomes, two additional enzymes with recognition sites of only four bases, namely Hae III and Msp I, were also needed. DNA differences obtained with these enzymes were recorded as 'genome subtypes' (e.g. D2a, 2b).

Six cases of prolonged shedding of AV 1 and five cases of AV 2 were studied

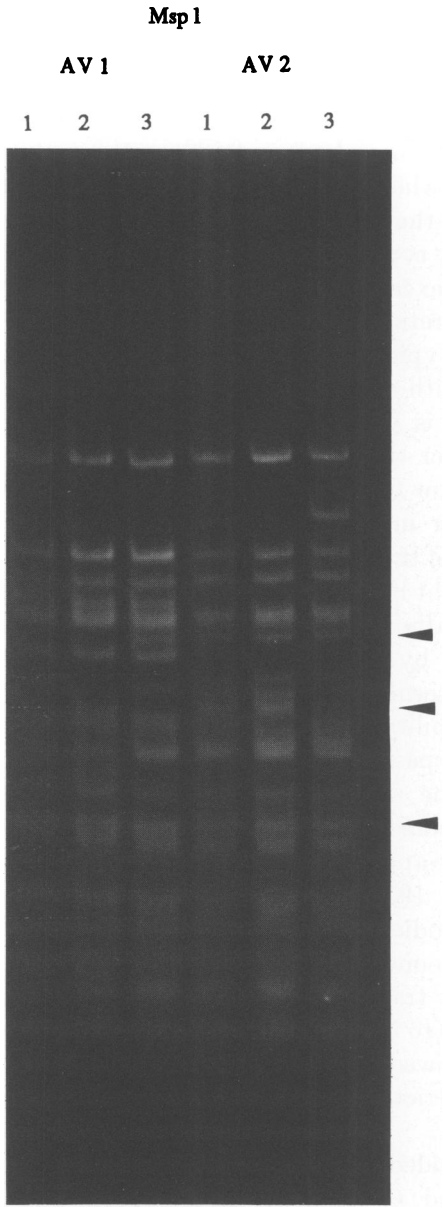

Fig. 3. DNA restriction patterns of AV 1 and AV 2 prototypes (lane 1) and deviating restriction patterns (see Table 1) with Msp I. Arrows point to small differences between the fragment patterns 1 and 2 of AV 2.

(Table 2). Viruses were obtained from faecal extracts for up to 515 days. The genome types of early, middle, and late isolates corresponded in all instances.

All restriction patterns observed with the seven standard enzymes in these isolates and in Ad V 1 and 2 prototype are shown in Figs 1 and 2. The genome type D10 of AV 1 and D2 of AV 2 could be subdivided into genome subtypes by either Hae III or Msp I or both (see Fig. 3 for Msp I).

In view of the high genetic variability of subgenus C AV (Wigand & Adrian,

1986), our results indicate that the 11 children studied were indeed persistently infected. Reinfection would almost certainly have been by a different genome type and this would have been revealed by DNA restriction analysis. It should be noted, that several of the observed genome types, notably D4 and D10 of AV 1 and D2 of AV 2, may be found frequently in the population (unpublished results). However, when the two additional endonucleases with four-base recognition sites and thus with increased discriminating power were applied, two of these three genome types were split into subtypes (Table 2). Even so the viruses from early and late excretion were identical. Brandt, Wasserman & Fox (1966) found prolonged excretion (about 300 days) of AV 5 in children. These isolates were serologically atypical but remained unchanged in early and late isolates, which is in accord with our results.

Another question is whether true intermittent excretion (recrudescence) of AV occurs or whether the excretion is continuous, but the amount of virus at certain times is too low for virus isolation (Wigand, 1978). This question is as yet undecided. A further intriguing question is the location of the persistent AV infection in sections of the intestinal epithelium, Peyer's patches or elsewhere. One way to resolve it might be an immunohistochemical or *in situ* hybridization study of infants' intestine after accidental death. Another possibility would be that the infections may arise by reactivation of persistent infections of the pharyngeal adenoid tissue (Wasserman, August & Plotkin, 1988).

Despite the variability of subgenus C adenoviruses, the genome stability of individual strains on passage in cell culture (Adrian *et al.* 1986) is high. The same is now found for the *in vivo* situation. Although we found no evidence for reinfection with the same serotype, cases with reinfection may nevertheless occur. Protective immunity apparently does not protect completely against reinfection (Fox, Hall & Cooney, 1977), and some infants were observed, in whom a titre rise of neutralizing antibodies occurred during late intermittent excretion (Fox *et al.* 1969; Fox, Hall & Cooney, 1977).

For the respiratory tract, a prolonged or intermittent excretion may also occur, if only for periods up to 55 days (Pacini, Collier & Henderson, 1987). One-third of the infants tested showed a late antibody rise. The isolates, however, had not been studied by DNA restriction analysis.

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