

GENOMIC CHARACTERIZATION OF ADENOVIRUS SEROTYPE 7 ISOLATED IN BRAZIL FROM ACUTE RESPIRATORY DISEASE PATIENTS DURING THE PERIOD FROM 1980 TO 1991

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SUMMARY

Forty isolates of adenovirus type 7 were analyzed by restriction enzyme digestion with *Bam*HI, *Sma*I, *Eco*RI and *Hind*III. These isolates were obtained from acute respiratory disease patients during the years 1980 to 1991. Only two genomic types were found: Ad7b and Ad7e, with Ad7b (87.5%) being more frequent than Ad7e (12.5%). The genomic type Ad7e appeared in the years 1980, 1981 and 1983. Ad7b appeared in 1982 and it was the only genomic type found from 1984 to 1991. Both genomic types were responsible for lower (LRTI) and upper (URTI) respiratory tract infection, but the proportion LRTI/URTI is higher for Ad7b (25/6) than for Ad7e (1/4).

KEYWORDS: Adenovirus type 7; Isolation; Genomic analysis.

INTRODUCTION

Adenovirus infections have been studied in Brazil since they were first described in 1953²⁰. The commonest types isolated from acute respiratory infections (ARI) in children have been, like elsewhere²¹, serotypes 1, 2, 5 and 7^{17,23,24}. During a previous study of Ad7 isolates obtained during 1980 and 1981 from North and Southeast Brazil only one genomic type was found, namely Ad7e, and this genomic type was not found in any other countries²⁷. Subsequently, it was shown by LI & WADELL¹⁵ that Ad7e had occurred before in Australia but had been replaced by genomic type Ad7b. The genomic type Ad7b was predominant in many countries, mainly in Europe²⁷. Another study of adenovirus type 7 isolates from Argentina described a new genomic type namely Ad7h¹⁹. This genomic type Ad7h was found to be prevalent among isolates from Argentina and Chile^{12,13} and latter also for isolates from Uruguay¹⁴. This paper deals with the results obtained by genotyping of Ad7 isolated in Brazil during the period between 1980 to 1991 from patients showing ARI, classified as upper (URTI) and lower (LRTI) respiratory tract infections on clinical grounds^{17,22,23}.

MATERIALS AND METHODS

Adenovirus isolates

Between 1980 and 1991, we isolated 208 adenovirus strains, and among those 40 were classified as Adenovirus type 7 by seroneutralization using standard sera kindly provided by

VRD/CPHL, U.K. From these 40 Ad7 isolates, 36 were from patients living in Rio de Janeiro State, being 35 children under five years age and one nurse working in contact with some of these patients. Two of these specimens were isolated from the same patient during different periods of his disease. All patients showed ARI and were classified as LRTI (26) and URTI (10). For three of these patients conjunctivitis was also present. Diarrhoea was also reported for four of those patients. Four other Ad7 isolates were from two adults showing URTI and rash living in the South of the country (Rio Grande do Sul) and two one year old children showing LRTI living in the North of the country (Pará). All specimens were isolated and identified as adenovirus in Hep2 cells, using indirect immunofluorescence with an anti-hexon guinea pig serum and anti-guinea pig IgG FITC conjugate; before being serotyped by neutralization using standard sera and procedures⁸.

Adenovirus type 7 standard genotypes

Ad7p, Ad7a, and Ad7b were obtained from VRD/CPHL, U.K. Ad7e was the original strain isolated in Brazil²⁷, and Ad7h was the original strain isolated in Argentina¹⁹.

DNA preparation and restriction nucleases digestion

Viral DNA was extracted from infected Hep2 cells using the method described by HIRT⁹ and modified by WADELL & DE JONG²⁹. DNAs were digested by restriction enzymes using

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protocols recommended by manufactures (Bethesda Research Laboratory, Boehringer Mannheim GmbH and Sigma Chemical Company, USA). Enzymes used were *Bam*HI, *Sma*I, *Hind*III, *Eco*RI, *Bg*III and *Xba*I. Fragments were analysed in 1 or 2% agarose gels by horizontal electrophoresis in TBE buffer. After electrophoresis the gels were stained with ethidium bromide (0.5 µg/ml) and then visualized with U.V. transilluminator. Photographs were obtained using Polaroid 665 or 667 films. The genome type classification system used was that described by LI & WADELL¹⁵.

Restriction maps used for comparison

The choice of restriction enzymes was based on the sites distributed within the adenovirus genome. Restriction maps for Ad7p and Ad7b with *Bam*HI, *Bc*III, *Bst*EII, *Eco*RI, *Hind*III, *Sma*I, *Hpa*I and *Xba*I; for Ad7a with *Bam*HI, *Hind*III and *Bg*III and for Ad7e with *Bst*EII, *Eco*RI, *Hind*III, *Sma*I, *Hpa*I and *Xba*I were obtained from ADRIAN et al.², LI & WADELL¹⁵, NIEL et al.¹⁹ and WADELL et al.²⁶.

Location of transcription and translation regions for Ad7b and Ad7e

Data about 0 to 30% and 70 to 100% of Ad7 genome were taken from VAN ORMOND & GALIBERT²⁵, for the remaining 30 to 60% we used Ad2 genome⁴ and for the region between 67 to 69% we use the Ad5 map²⁵.

RESULTS

DNA preparations from forty Ad7 isolates were first analysed by digestion with *Bam*HI. Thirty-five were classified as Ad7b and five as Ad7e (Table 1). All strains initially classified as Ad7e were confirmed as Ad7e by digestion with *Sma*I, *Hind*III and *Eco*RI. Thirty-six strains analysed were from Rio de Janeiro, collected during the period between 1980 and 1991 (except for the years 1985 to 1987 and 1989, when no adenovirus isolated was serotyped as Ad7). From these 36 strains, 31 were classified as Ad7b and 5 as Ad7e. Figure 1 shows the temporal distribution of Ad7b and Ad7e strains isolated in Rio de Janeiro. During the years 1980 and 1981, Ad7e was the only genotype found. No Ad7e were found among 1982 strains. During the year 1983 only one of 12 strains analysed was Ad7e. Subsequently no further Ad7e strains were found; all strains were classified as Ad7b. Four strains were analyzed from other Brazilian states, 2 of these were from Pará (North Brazil) collected during 1983 and 2 were from Rio Grande do Sul (South Brazil) collected during 1984. These were also classified as Ad7b. Among the 36 patients studied from Rio de Janeiro, twenty-six had LRTI and 10 URTI. Four strains obtained from the 10 patients showing URTI were collected during 1980(2) and 1981(2). All four strains were classified as Ad7e. The other Ad7e found was during 1983 and it was isolated from a patient showing LRTI. During 1983 a larger number of specimens was investigated. Most adenovirus isolates were serotyped as Ad7 and sub-classified as Ad7b (11/12). Four strains were from patients showing also conjunctivitis, three of these were classified as Ad7b, and one as Ad7e. Two strains

were from patients with diarrhoea; and both were classified as Ad7b. Two isolates from the same patient in different periods of disease, were both classified as Ad7b. They were collected in a 5 day interval during which the disease evolved from URTI to LRTI. Twelve of the analysed strains were isolated from patients receiving medical care in paediatric wards of the same hospital during the years 1983 and 1984. Ten of those patients were considered as having nosocomial infections because the disease they showed in admission was not classified as URTI or LRTI. Two of these children died. One of the strains analyzed was from the nurse that had conjunctivitis and URTI. All these 12 strains were classified as Ad7b.

The comparison of the restriction maps of genome types Ad7b and Ad7e with the prototype Ad7p is shown in figure 2a and 2b. Thirteen changes were identified for Ad7b and 19 for Ad7e compared with Ad7p. Table 2 shows the comparison between Ad7b and Ad7e with respect to these mutations. The Ad7e genome shows more mutation sites in region E1 (E1A and E1B), E2 (E2A and E2B), E3, DNA binding protein sequence and DNA polymerase sequence. The Ad7b genome shows two mutations in the E4 region, which were not observed for Ad7e.

TABLE 1
Clinical and epidemiological characterization of patients from whom were isolated 40 Brazilian Ad7 strains.

Brazilian Region	Number of Isolates	Period	Age range		Clinical Classification	Ad7 Genotype
			Child	Adult		
Southeast (RJ)	36	1980-1991	35	1	URTI (10) LRTI (26)	Ad7e (4)
						Ad7b (6)
						Ad7e (1) Ad7b (25)
South (RS)	2	1984	0	2	URTI	Ad7b
North (PA)	2	1983	2	0	LRTI	Ad7b

TABLE 2
Comparison of genomic maps from types 7b and 7e in relationship to the Ad7p map.

Mutated sites	Adenovirus genomic types	
	Ad7b	Ad7e
Structural regions (hexon, penton, fiber)	04	04
E1 Region (E1A and E1B)	02	04
E2 Region (E2A and E2B)	02	05
E3 Region	01	02
E4 Region	02	00
DNA binding protein sequence	00	01
DNA polymerase sequence	00	01
TATA box sequence (E1A)	01	01
Total number of mutations	13	19

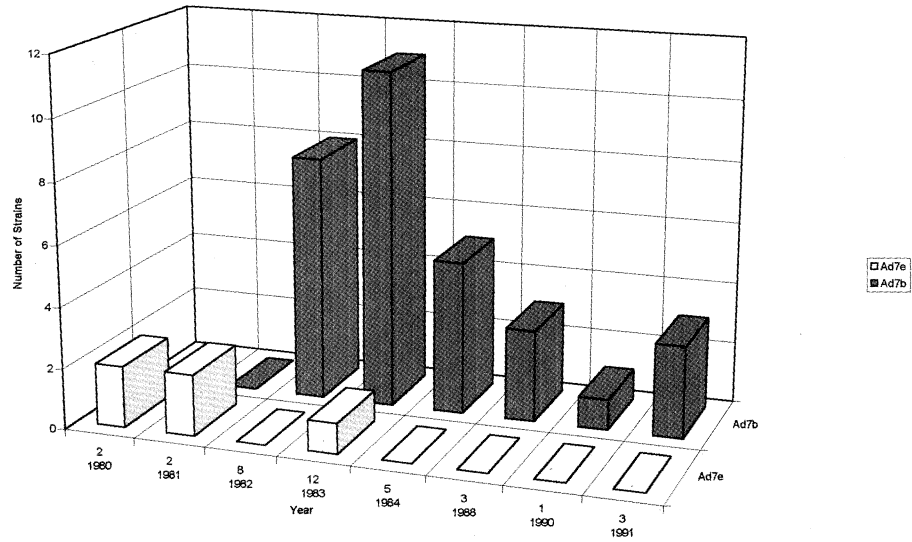


Fig. 1 - Distribution of Ad7 genotypes in Rio de Janeiro from 1980 to 1991

Fig. 2A - *Bg*III restriction maps for Ad7a and Ad7e genotypes

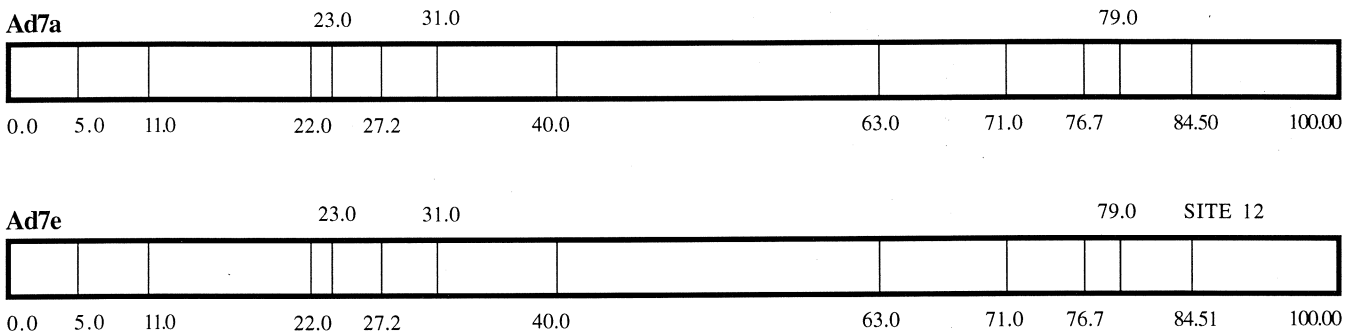


Fig. 2B - *Bg*III restriction fragments for Ad7a and Ad7e genotypes

Ad7a	Ad7e
A 8100	A 8100
B 5400	B 5400
C 3700	C 3700
D 3220	D 3220
E 2720	E 2720
F 2470	F 2470
G 2380	G 2380
H 2300	H 2340
I 1880	I 1880
J 1550	J 1550
L 1270	L 1270
M 1020	M 1020
N 630	N 630

DISCUSSION

WADELL & VARSAYI³⁰ showed that Ad7 isolates could be clustered in three genomic groups. These were representatives of the prototypes strain (Ad7p); representative of the vaccine strain and strains isolated from healthy carriers or showing minor symptoms (Ad7a); or representative strains isolated from Swedish patients showing severe systemic infections (Ad7b). The distribution of the different genotypes of Ad7 was also studied using strains isolated from different European countries during the years from 1958 to 1981²⁸. Four genomic types were found, namely Ad7p, Ad7a, Ad7b and Ad7c. The distribution of these genotypes was not homogeneous since Ad7a, Ad7b and Ad7c were found among isolates obtained from 1958 to 1972 whereas all isolates obtained from 1972 to 1981 were classified as Ad7b. Three additional genomic types were found when isolates from the 5 different continents were analysed: Ad7d,

Ad7e and Ad7f²⁷. Although Ad7b was shown to be predominant worldwide, some geographic and temporal distributions of these genotypes were seen. Ad7d occurred in China during 1981 and it was predominant over a period of 11 years¹⁶. From Brazil, all 8 virus analyzed were Ad7e and Ad7f was found among isolates (1 in 22) obtained from Australia²⁷. Working with 336 Ad7 isolates from different countries and with 12 restriction endonucleases, LI & WADELL¹⁵ recognized 15 different genome types and studied the relationship among them by analysing the percentage of comigrating restriction fragments (PCRf). The Brazilian strains were confirmed as Ad7e and it was shown that this genomic type had also circulated in Australia. ADRIAN et al.¹ also reported a predominance of Ad7b in isolates from USA, Germany and Sweden, during the period from 1961 to 1985. Other isolates obtained in South America were analysed by NIEL et al.¹⁹. Another genotype, namely Ad7h, was described circulating in Argentina. KAYON et al.^{10,11,12,13,14} extensively studied Ad7 isolates obtained during the years 1984 to 1990, in Argentina, Chile and Uruguay, and found Ad7c, Ad7b and Ad7h in Argentina and Chile. Ad7c was predominant in both South American countries until 1986 when Ad7h appeared, which became predominant. Adenovirus disease is considered severe in Argentina and it is frequently associated with fatal cases⁵ that it was correlated with the Ad7h genotype circulating there. We are now reporting genomic analysis for forty Brazilian Ad7 isolates. Confirming the work done by WADELL et al.²⁷, the only genotype found during the years 1980 and 1981 was Ad7e. All these four strains were isolated from patients showing URTI. The Ad7e found during 1983 was, however, isolated from a child diagnosed with LRTI. As occurred in USA (1962), Europe (1969) and Australia (1975) there was also a shift to Ad7b during 1982 in Brazil. No other genomic type was found from ARI since then, excluding the one Ad7e isolated in 1983. This is also the case for virus isolated from North and South Brazil during 1983 and 1984. Studies done in Brazil about Ad7 isolated from stools of children living in Southeast, Rio de Janeiro¹⁸ and São Paulo⁷, or Central Brazil, Goiás (J.P.G. Leite, not published), are in agreement with our findings. The genomic type Ad7h appeared in Argentina and Chile during 1986 and it has become prevalent in these South American countries since then. We could not detect any Ad7h among our Ad7 strains, although Ad3h, which is also found in Argentina¹⁹, was detected in Rio de Janeiro during 1990. Analysing our present results we were not able to correlate Ad7 genomic type and disease severity because of the low numbers of strains studied, and also because both Ad7e and Ad7b have occurred in both groups of patients with URTI and LRTI. Ad7e is closely related to Ad7a with a PCRf reaching 94%¹⁵. The PCRf analysis for the 15 Ad7 genotype shows that most of them belong to cluster 3, excluding Ad7p and Ad7p1 (cluster 1) and Ad7g (cluster 2). As Ad7a is found to be less pathogenic than other Ad7 genomic types and it is similar to Ad7e^{15,27,30} we analysed the differences among Ad7b, Ad7e and Ad7p restriction maps. Several mutations were located in regions considered important for virus replication including E1A and E1B, E2A and E2B, DNA polymerase, DNA binding protein and E4 regions. GINSBERG et al.⁶ working with adenovirus mutants defective in the DNA binding protein region has shown that these mutants are less effective in causing pneumonia in animals than the wild type virus. It seems that for

the development of pneumonia, the accumulation of structural proteins in lung tissue is needed. Therefore mutations affecting virus replication will lesser the appearance of lung lesions. The result of the analysis of the Ad7e restriction map could theoretically explain why Ad7e is less likely than Ad7b to be pathogenic.

Respiratory syncytial virus is the major viral agent isolated from ARI in less than five years old children in Rio de Janeiro²². Adenovirus is the second most frequently found¹⁷. Similar results were described in Argentina³. In a previous study during 1980-1981²³ involving outpatients living in a Rio de Janeiro shanty town, adenovirus was the most frequently virus isolated from children showing URTI. It was less important among children with LRTI, in which it had the same prevalence as enterovirus. Adenovirus types 1 to 7 were found, but type 5 was predominant. During these two years Ad7e was the only genomic type found in Brazil²⁷ (Figure 1). During the next four years (1982-1985) we isolated 85 adenovirus strains. The most frequent serotypes found were Ad2 (29%) and Ad7 (20%). Among children receiving medical care in paediatric and emergency wards Ad7 represented 27% and Ad2 23% of the isolates. Among outpatients Ad2 (37%) and Ad1 (27%) were more frequently found¹⁷. This increased occurrence in Ad7 in LRTI patients could be a result of the appearance of Ad7b during 1982. From 1986 to 1991, adenovirus type 2 was the predominant serotype among 87 isolates. During the years 1985, 1986, 1987 and 1989 none of the adenovirus isolated belonged to serotype 7. From Figure 1 it can be seen that during 1983 there is a high predominance of Ad7 strains, but this includes 10 strains related to a nosocomial outbreak. The low prevalence of Ad7 in subsequent years could be due to herd immunity as has been shown for the Japanese population and Ad7p²⁷. The shift from Ad7e to Ad7b which occurred during 1982 to 1984 modified the frequency of severe disease associated with adenovirus type 7 in Rio de Janeiro. From table 1 it can be seen that the proportion of LRTI/URTI is higher for Ad7b (25/6) than for Ad7e (1/4). It is important to establish if this also occurred in other areas of Brazil in the same way. This will help better understanding of the correlation of Ad7b genomic type and strain pathogenicity, as has been recognized for virus circulating in European countries³¹.

RESUMO

Caracterização genômica de adenovírus sorotipo 7 isolados no Brasil de pacientes com infecção respiratória aguda, durante os anos de 1980 a 1991

Quarenta amostras de Adenovírus tipo 7 foram analisadas por digestão com as enzimas de restrição *Bam*HI, *Sma*I, *Eco*RI e *Hind*III. Estas amostras foram coletadas de pacientes apresentando doença respiratória aguda durante os anos de 1980 a 1991. Somente dois tipos genômicos foram encontrados: Ad7b e Ad7e, sendo que Ad7b (87,5%) foi mais frequente que Ad7e (12,5%). O tipo genômico Ad7e apareceu nos anos 1980, 1981 e 1983. Ad7b apareceu durante 1982 e seguiu sendo o único tipo genômico encontrado de 1984 a 1991. Ambos os tipos

genômicos foram responsáveis por infecções do trato respiratório superior (ITRS) e inferior (ITRI), porém a proporção ITRI/ITRS é mais alta para Ad7b (25/6) que para Ad7e(1/4).

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