GENOTYPING OF URUGUAYAN HUMAN ADENOVIRUS ISOLATES COLLECTED BETWEEN 1994 AND 1998

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Summary. – Adenoviruses are one of the most frequent causative agents of acute lower respiratory infections in infants and young children. Twenty-three adenovirus isolates from nasopharyngeal aspirates of children hospitalized for acute lower respiratory infections in Uruguay between 1994 and 1998 were studied by restriction enzyme analysis. The genomic analysis showed that 60.9% (n = 14) of isolates belonged to the species *Human adenovirus C* (*HAdV-C*) and 31.9% (n = 9) to the species *Human adenovirus B* (*HAdV-B*). Whereas some isolates could be classified according to the published profiles into genotype or genomic variant, others displayed migration patterns not allowing classification. Eight isolates (89%) of *HAdV-B* corresponded to the Ad7h genotype that has been associated with severe and fatal pneumonia and necrotizing bronchiolitis in children in South America. The isolates of *HAdV-C* showed a great variability in accordance with the data published earlier.

Key words: human adenoviruses; genotyping; restriction analysis

Introduction

Human adenoviruses (the *Mastadenovirus* genus, the *Adenoviridae* family) (van Regenmortel *et al*., 2000) belong to the most frequent causative agents of acute lower respiratory infections in infants and young children (Horwitz, 2001; Brandt *et al*., 1969). In developing countries, adenoviruses cause serious illness and death in childhood (Pio *et al*., 1984; Berman, 1991). Among them, those classified within the species *HAdV-B* (serotypes or simply types 3 and 7), *HAdV-C* (types 1, 2 and 5) and *HAdV-E* (type 4) are regarded as the cause of respiratory diseases (Sharp and Wadell, 1995). In South American countries, adenoviruses were found to be the second major group of viruses after Respiratory syncytial virus which infect the respiratory tract of children (Suarez *et al*., 1988a,b; Avila *et al*., 1989, 1990; Nascimento *et al*., 1991; Kajón *et al*., 1996).

Restriction analysis performed on 23 adenovirus isolates from Argentina, Chile and Uruguay between 1991 and 1994 revealed that, whereas a majority of them belonged to the *HAdV-B* species, the remaining ones were classified mostly as *HAdV-C* and rarely as *HAdV-E* (Kajón *et al*., 1996).

In this report we present the results of genotyping by restriction analysis of 23 adenovirus isolates originating from children with acute lower respiratory infections from Uruguay between 1994 and 1998.

Materials and Methods

Virus isolation was performed on HEp-2 cells from nasopharyngeal aspirates collected from children under five years with acute lower respiratory diseases between 1994 and 1998 in Uru-

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Abbreviations: CPE = cytopathic affect; IFA = immunofluorescence assay; *HAdV-B = Human adenovirus B; HAdV-C = Human adenovirus C; HAdV-E = Human adenovirus E*

guay. The cells were grown in Eagle´s Minimal Essential Medium containing 10% of fetal bovine serum, 30 mg/ml glutamine, 100 µl peniciline and 50 µl gentamicine. Upon appearance of cytopathic effect (CPE), the cultures were frozen.

Immunofluorescence assay (IFA). Identification of adenovirus infection was performed by an indirect IFA (Ballew *et al*., 1984) on infected culture cells showing CPE using commercial monoclonal antibodies (Chemicon International, Inc.) conjugated with fluorescein.

Viral DNA extraction. Each viral isolate was inoculated onto 75–80% confluent monolayers of HEp-2 cells in 75 cm^2 flasks. Upon appearance of an extensive CPE, the cells were harvested and the DNA was extracted according to Shinagawa *et al*. (1983) with modifications.

Restriction analysis of viral DNA and agarose gel electrophoresis. One µg of viral DNA extracted from each sample was digested with 10 U of *Bam*HI, *Sma*I and *Bgl*II under conditions specified by the manufacturer (Promega). The digested viral DNAs were loaded onto 0.7% agarose gel containing 0.25 µg/ml ethidium bromide and run at 80 V for 3 hrs in TBE buffer pH 8.0. Restriction profiles were visualized under UV light and photographed. The resulting restriction profiles were assigned to corresponding virus species and genotypes according to the literature data.

***Sma*I**

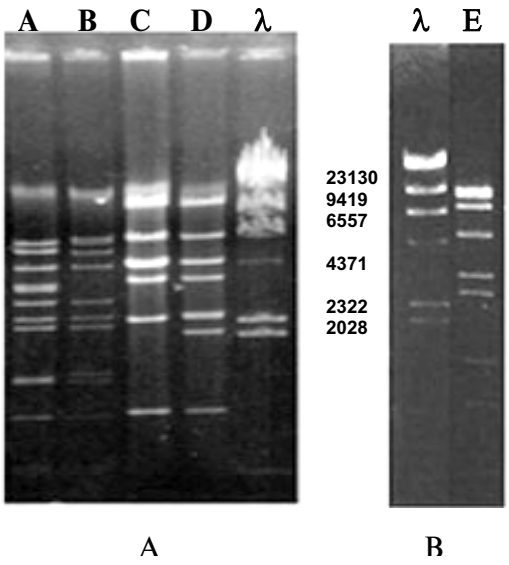

Fig. 2

Restriction profiles of standard genotypes of some human adenoviruses

Agarose gel electrophoresis, *Sma*I digestion. (A). Genotypes Ad5 (lane A), Ad1 (lane B), Ad7b or Ad7d2 (lane C), Ad7h (lane D). (B). Genotype Ad2 (lane E). DNA size markers (lane λ).

***Bam*HI**

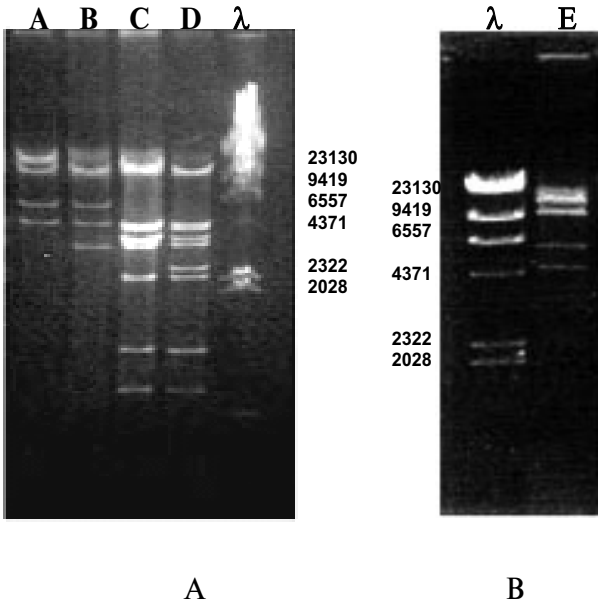

Fig. 1

Restriction profiles of standard genotypes of some human adenoviruses

Agarose gel electrophoresis, *Bam*HI digestion. (A). Genotypes Ad5 (lane A), Ad1 (lane B), Ad7b or Ad7d2 (lane C), Ad7h (lane D). (B). Genotype Ad2 (lane E). DNA size markers (lane λ).

***Bgl*II**

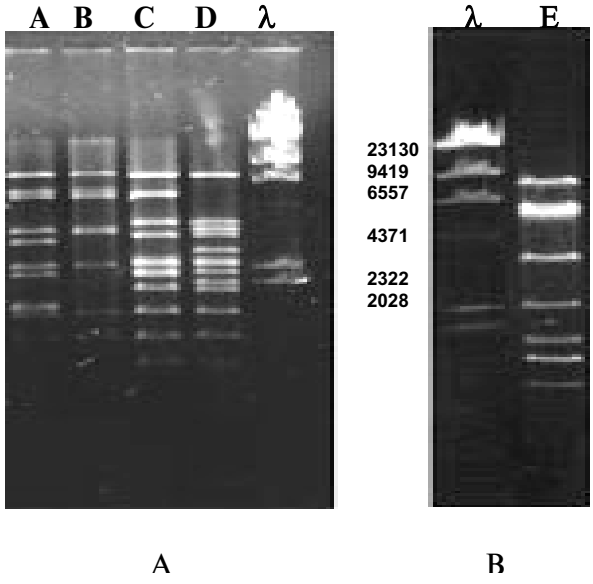

Fig. 3

Restriction profiles of standard genotypes of some human adenoviruses

Agarose gel electrophoresis, *Bgl*II digestion. (A). Genotypes Ad5 (lane A), Ad1 (lane B), Ad7b or Ad7d2 (lane C), Ad7h (lane D). (B). Genotype Ad2 (lane E). DNA size markers (lane λ).

Table 1. Genotyping of human adenovirus isolates

Species	Genotype of genome variant	No. of isolates	Years of circulation
HAdV-B			
n = 9 (31.9%)	Ad7h	8	94, 95, 96, 97, 98
	Ad7b or Ad7 d2[1]	1	98
HAdV-C			
n = 14 (60.8%)	Ad1D1[2], AV1 New II[3]	4	95, 96, 97, 98
	AV2 New IV[4], Ad2p1[5]	3	96
	AV2 New IV[6]	6	97, 98
	AV5 New V	1	98

[1]Based on *Bgl*II profile.
[2]Based on *Bam*HI profile.
[3]Based on *Sma*I and *Bgl*II profiles.
[4]Based on *Bgl*II profile.
[5]Based on *Sma*I profile.
[6]Based on *Bam*HI and *Bgl*II profiles.

Results and Discussion

A total of 23 epidemiologically unrelated adenovirus isolates from nasopharyngeal aspirates from children under 5 years of age hospitalized with acute lower respiratory disease between 1994 and 1998 in Uruguay were genotyped by digestion of viral DNAs with *Bam*HI, *Sma*I and *Bgl*II. The resulting restriction profiles were assigned to corresponding genotypes according to the literature data.

The restriction analysis showed that 60.8% of the analyzed isolates belonged to *HAdV-C* and 39.1% to *HAdV-B* (Table 1). These results were in accordance with an earlier report from Brazil (Kajón *et al.*, 1999), where the incidence of *HAdV-C* was 64.1% and that of *HAdV-B* was 35.8%, but in contrast to other reports from South America (Kajón *et al.*, 1996; Videla *et al.*, 1999; Larrañaga *et al.*, 2000). However, no conclusion can be drawn due to the low number of isolates analyzed.

Of the nine isolates (39.1%) identified as *HAdV-B* eight corresponded clearly to the genotype Ad7h (Figs. 1–3, lanes D) (Hashido *et al*., 1999; Kajón *et al*., 1996; Videla *et al*., 1999, Erdman *et al*., 2002).

Single isolate displayed *Bam*HI and *Sma*I restriction profiles with a single fragment of about 2.322 bp instead of two expected for the genotype Ad7h (Figs. 1 and 2, lanes C) but a *Bgl*II restriction profile (Fig. 3, lane C) fully matching the genotype Ad7b or Ad7d2 (Li and Wadell, 1988; Hashido *et al*., 1999; Erdman *et al*., 2002). Ad7h turned out to be the genotype associated with severe fatal pneumonia and necrotizing bronchiolitis in small children in South America (Kajón and Wadell, 1992; Kajón *et al.,* 1994, 1996; Larrañaga *et al*., 2000). In this study, 88.8% of the isolates belonging to *HAdV-B* were identified as the Ad7h genotype.

Of the fourteen isolates belonging to *HAdV-C* one was genotyped as Ad5 New V genomic variant (Figs. 1–3, lanes A) using all the three restriction enzymes. The other thirteen isolates showed a marked variability. Four isolates were genotyped as Ad1D1 (Fig. 1, lane B) based on *Bam*HI profile (Kajón *et al.*, 1996; Videla *et al.*, 1999) but as AV1 New II genomic variant according to *Sma*I and *Bgl*II profiles (Figs. 2 and 3, lanes B).

Three isolates were assigned to AV2 New IV genomic variant (Kajón *et al.*, 1996) based on *Bgl*II profile but to Ad2p1 according to *Sma*I profile (Li *et al.*, 1999). The *Bam*HI digestion of DNAs of these isolates could not be evaluated due to obscure profiles.

Six isolates were genotyped as AV2 New IV genomic variant according to *Bgl*II and *Bam*HI profiles (Figs. 1–3, lanes E). However their *Sma*I profiles did not match the expected ones (Kajón *et al*., 1996) (Fig. 2, lane E).

The higher genome variability of the isolates of *HAdV-C* compared to those of *HAdV-B* was in accord with the data of other authors (Avila *et al*., 1990; Nascimento *et al*., 1991). Several variants were found among the fourteen isolates belonging to *HAdV-C*. Thus, of fourteen Uruguayan isolates of *HAdV-C* thirteen did not match the profiles reported earlier for this virus species (Table 1). On the other hand, of nine Uruguayan isolates of *HAdV-B* only one did not match the expected profiles.

The data obtained in this study show the presence of human adenoviruses of species B (*HAdV-B*) and C *(HAdV-C*) isolated from epidemics between 1994 and 1998 in Uruguay. Although just a low number of serum samples as well as virus isolates (n = 23) was analyzed, genetic variability of human adenoviruses reflected in different species, genotypes and genotype variants has been demonstrated.

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