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Genome Analysis of South American Adenovirus Strains of Serotype 7 Collected over a 7-Year Period

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DNA restriction analysis was carried out on a sample of 212 epidemiologically unrelated adenovirus (Ad) strains of serotype 7 isolated in Chile, Uruguay, and Argentina from nasopharyngeal aspirates of children with acute lower respiratory disease between 1984 and 1990. The identified genome types were Ad7b (n = 12), Ad7c (n = 21), and Ad7h (n = 179). The analysis of the occurrence of the three genome types throughout the study period revealed that Ad7c was the predominant virulent variant in 1984 and indicated that a shift to genome type 7h took place in 1986. Ad7b cocirculated with the other genomic variants at a relatively low frequence.

Adenoviruses (Ad) have been shown to play an important role in the etiology of severe acute respiratory disease, particularly in infants and young children (2, 10). Among the serotypes more frequently isolated from patients with respiratory disease, the members of subgenus B:1, and specially Ad serotype 7 (Ad7), have been associated with clinical manifestations of considerable severity, with fatal outcomes or residual lung damage (9, 12, 13).

Since the isolation of prototype strain Gomen in 1958, several different genome types of Ad7 have been identified by use of restriction enzymes with 6-nucleotide recognition cleavage sites and their worldwide distribution has been reported (7, 14). However, the molecular epidemiology and clinical impact of Ad7 respiratory infections in South America are still very poorly known. In this article we report the results of genome typing of a collection of 212 epidemiologically unrelated isolates of Ad7 collected in Argentina, Chile, and Uruguay between 1984 and 1990.

The studied sample consisted of 212 strains of Ad7 recovered from nasopharyngeal aspirates of children in Chile (n = 170), Uruguay (n = 8), and Argentina (n = 34) between 1984 and 1990. All patients were hospitalized for lower acute respiratory disease in the cities of Santiago (Chile), Montevideo (Uruguay), and Buenos Aires (Argentina). Acute lower respiratory disease was defined as an illness resulting in two or more of the following signs and symptoms: tachypnea, cough, rales, chest indrawing, wheezing, and stridor.

Virus strains were propagated in monolayers of HEp-2 or A-549 cells incubated in Eagle minimum essential medium supplemented with 2% fetal calf serum. When extensive typical adenovirus cytopathic effect was evident, intracellular viral DNA was extracted by the method of Shinagawa et al. (11).

Aliquots containing 1 to 2 μg of viral DNA were digested with 10 to 15 U of different endonucleases under conditions specified by the manufacturers. DNA digests were analyzed in 0.8 to 1.2% agarose horizontal gels. *Cfo*I digests were analyzed in mixed 1% SeaKem ME– 2% Nu Sieve (FMC Bioproducts, Rockland, Mass.) horizontal agarose gels. Bands were visualized by staining with 0.25 μg of ethidium bromide per ml and inspection under UV light. Stained gels were photographed on Polaroid Land film type 57. Genome type assignments were made by comparing the resulting profiles with the restriction patterns of prototype and more recently circulating genomic variants either run in parallel or taken from the literature. The genome type denomination system described by Li and Wadell (7) was used.

Restriction with *Bam*HI and *Sma*I allowed the identification of three different genome types. Eight isolates (5.7%) corresponded to genome type 7b, 21 isolates (9.9%) were identified as genome type 7c, and 179 isolates (84.4%) corresponded to genome type 7h. The corresponding *Bam*HI restriction profiles are shown in Fig. 1. All strains in the three genome types were further analyzed by restriction with *Bcl*I, *Bgl*II, *Bst*EII, and *Hin*dIII. Only 3 of 12 Ad7b strains and 2 of 179 Ad7h strains exhibited a deviant *Bgl*II profile. The corresponding restriction patterns are shown in Fig. 2. No variations were recorded after digestion with the other endonucleases. All analyzed strains of Ad7b, Ad7c, and Ad7h exhibited identical profiles after diges-

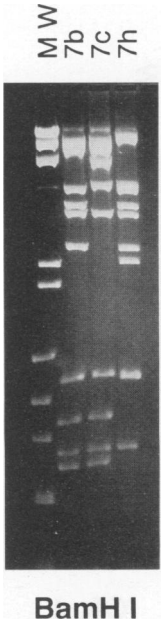

FIG. 1. *Bam*HI restriction profiles of Ad7b, Ad7c, and Ad7h. MW, molecular weight ladder (λ *Hin*dIII plus ϕX *Hae*III DNA).

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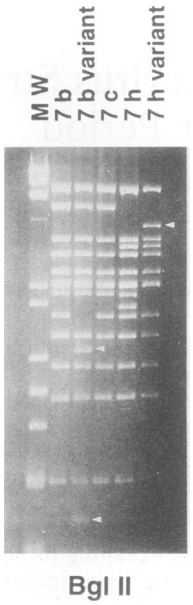

FIG. 2. *Bgl*II restriction profiles of Ad7 genomic variants. MW, molecular weight ladder (λ *Hin*dIII plus ϕX *Hae*III DNA).

tion with *Cfo*I, a restriction enzyme with a 4-base recognition cleavage site (GCGC). As shown in Fig. 3, Ad7b and Ad7c strains shared the same *Cfo*I pattern.

The temporal distribution of all 212 Ad7 analyzed strains is shown in Fig. 4. Ad7h is a newly identified genome type associated with lower respiratory infection that has so far been reported to circulate only in South America in association with very severe and fatal disease similar to that described for Ad7b infections (8). The remarkable homogeneity in the Ad7h *Cfo*I profiles reveals a great stability of this genome type throughout the 7-year period.

The analysis of the occurrence of the three genome types throughout the study period suggests that Ad7c was the

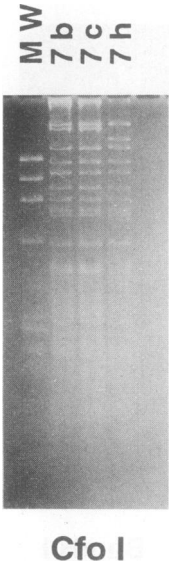

FIG. 3. *Cfo*I restriction profiles of Ad7b, Ad7c, and Ad7h. MW, molecular weight ladder (ϕX *Hae*III DNA).

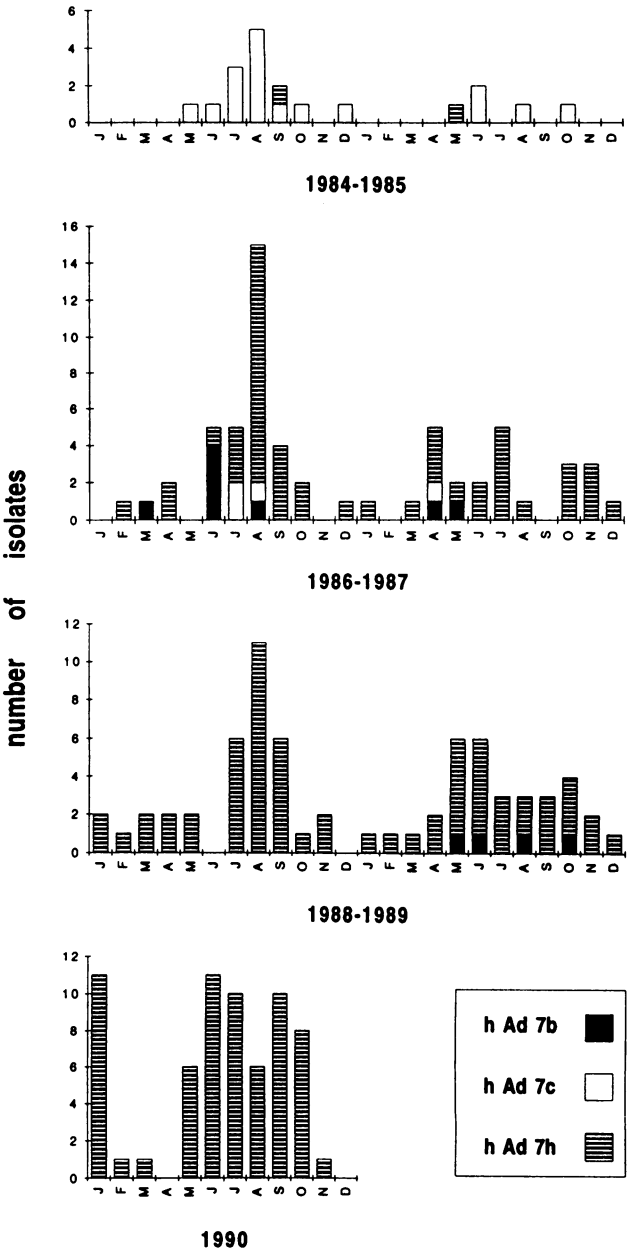

FIG. 4. Temporal distribution of Ad7 isolates of genome types 7b, 7c, and 7h (1984 to 1990).

predominant virulent DNA variant in 1984 and indicates that a shift to genome type 7h took place in 1986. Ad7b was found to cocirculate with the other two genome types in a relatively low frequency.

In Europe and Australia a shift was recorded from Ad7c to Ad7b, which is the currently predominant virulent genomic variant in those areas as well as in Brazil and possibly North America, where it has been reported to circulate during the last decade (1, 14, 15).

The occurrence of an Ad7 genome type shift has also been recorded in the former Soviet Union (3), from Ad7a to Ad7f.

Although the molecular bases of the observed changes remain to be investigated, it is noteworthy that the currently

predominant genome types circulating all over the world belong to the same cluster of homology.

By the analysis of the percentage of comigrating restriction fragments, Ad7h was previously found to be related to Ad7b (86%) and Ad7c (83%), although not as closely as the other members (Ad7a, Ad7d, Ad7e, and Ad7f) of the genomic cluster 3 (6).

The predominance of a few genome types constantly occurring over extended time periods is well known for human Ad's, and among the factors that may account for the substitution of a genomic variant by another, a greater capacity to spread and a higher virulence should be considered, although differences in pathogenicity have been difficult to prove.

South America, then, with the predominance of genome type 7h in Argentina, Chile, and Uruguay (4, 5) and Ad7b in Brazil (1) seems to have a unique molecular epidemiological picture like China and the former Soviet Union, where the predominant virulent types at present are 7d and 7f, respectively (3, 7). Previous studies (4, 5) suggest that there are no significant differences in the prevalence of Ad7 genotypes between Argentina and Chile.

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