Genome Type Analysis of Adenovirus Types 3 and 7 Isolated during Successive Outbreaks of Lower Respiratory Tract Infections in Children

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Adenovirus is an important cause of respiratory infections in infants and children. Fifty-one serotypes have been identified, and adenovirus type 3 (Ad3) and Ad7 have often been associated with outbreaks of severe respiratory tract infections. Each serotype can be further divided into genome types based on the patterns of digestion of their DNAs with restriction enzymes. DNA restriction analysis was performed with 56 strains of Ad3 and 98 strains of Ad7 by using 12 restriction enzymes recognizing 6 bp (BamHI, BclI, BglII, BgiII, BstEII, EcoRI, HindIII, HpaI, SalI, Smal, XbaI, and XhoI). The virus strains were isolated during outbreaks of lower respiratory tract infections in children during an 11-year period from 1990 to 2000 in Seoul, Korea. Among the Ad3 strains, seven genome types were identified; Ad3a and six novel types (Ad3a13, Ad3a14, Ad3a15, Ad3a16, Ad3a17, and Ad3a18). Multiple genome types cocirculated during outbreaks, and some of these were isolated during the 11-year observation period, while others were restricted to particular outbreaks. For Ad7, two genome types, Ad7d and Ad7l, the latter of which is a novel genome type, were identified. A shift in genome types occurred from Ad7d to Ad7l during successive outbreaks. Mortality was 3.6% among children with Ad3 infections and 18% among children infected with either of the Ad7 genome types. In conclusion, the data confirm that Ad3 genome types are more diverse than those of Ad7 and suggest that shifts of genome types may occur during successive outbreaks of Ad3 and Ad7.

Adenovirus is an important cause of respiratory tract infections (26, 36) and is responsible for 5 to 10% of lower respiratory tract infections (LRTIs) in infants and children. Among the 51 serotypes of human adenoviruses identified to date (4), about one-third are associated with human diseases. Adenovirus infections can occur endemically or as outbreaks. The serotypes frequently recovered from children with LRTIs are adenovirus type 1 (Ad1), Ad2, Ad3, Ad5, and Ad7. Ad1, Ad2, and Ad5 are usually isolated from endemic or sporadic cases, although epidemics with these types have been reported. Ad3, Ad7 and, less frequently, Ad21 are responsible for most epidemics of LRTIs in children and are also identified in sporadic cases.

Most adenovirus LRTIs are mild and indistinguishable from LRTIs caused by other respiratory viruses. However, adenoviruses can cause severe, often fatal pneumonia or bronchiolitis. Severe acute respiratory illness has been noted in association with Ad1 through Ad8, Ad19, Ad21, Ad35, and others, but outbreaks of severe infections in healthy children are most frequently reported with Ad7, followed by Ad3 and Ad21 (11, 16, 20, 29). Epidemics of severe acute respiratory illness caused by these adenovirus types have often been noted among infants of Asian descent and native populations in New Zealand, Canada, and northern Finland. It is unknown whether this severe form of respiratory illness in those populations is attributable to genetic differences, environmental factors such as socioeconomic status, crowding in a cold climate, or the virulence of viral strains.

Different genomic DNA patterns within each serotype can be identified by restriction enzyme analysis. These different restriction patterns have been designated genome types (31, 34). Genome types may vary by location and time of isolation and may be useful markers for epidemiological studies (1–3, 6–9, 15, 16, 18–22, 24, 27, 29–34). It has been suggested that some genome types may be associated with greater virulence (15, 16, 34). Therefore, analysis of genome types is of clinical as well as epidemiological importance.

Different systems used to classify or denominate genome types have been proposed. Among these, the system proposed by Li and Wadell (19) and subsequently modified by Li et al. (20) has been widely used. In their nomenclature system, the prototype strain is abbreviated “p,” and strains that have the same serotype but that have different BamHI patterns are designated “a,” “b,” “c,” and so forth. An Arabic numeral is added after “p,” “a,” “b,” “c,” and so forth to describe the different genome types distinguished by use of additional restriction endonucleases (e.g., Ad3p, Ad3p1, Ad3a, Ad3a5, and Ad7d2). Genotype A of Ad3 that have been identified to date are 3p, the prototype and 3a through 3x and their variants.
Genome types of Ad7 that have been identified are 7p and 7a through 7k and their variants.

The genomic diversity of adenoviruses has been a subject of many publications. To our knowledge, however, there have been limited reports on the genome types of Ad3 and Ad7 in successive outbreaks over an extended observation period in the same geographic region. In Korea, successive outbreaks of severe acute respiratory illnesses caused by Ad3 and Ad7 infections in children were recognized during the 1990s (11). This study was performed to characterize the genome types of Ad3 and Ad7 strains isolated during these successive outbreaks and to investigate the possible relationship between genome type and clinical severity of illness.

MATERIALS AND METHODS

Patients and viruses. Adenovirus strains isolated from nasal aspirates of infants and children who presented with clinical evidence of LRTIs at the Department of Pediatrics, Seoul National University Children’s Hospital, Seoul, Korea, during the period from November 1990 through July 2000 (11) were analyzed. Also included in the virological analysis were strains isolated from referred respiratory specimens obtained from patients with LRTIs and hospitalized in other hospitals. The viruses were kept frozen at −70°C. All but five adenovirus strains isolated during the study period were subjected to serotype determination, and all Ad3 and Ad7 strains were genome typed. For comparison, Ad7p (Gomen), Ad7A (St1058), and Ad3p (G.B.) strains obtained from the American Type Culture Collection (Manassas, Va.) and Ad3a, Ad7d (383), and Ad7d2 (Bal) reference strains obtained from the National Institute of Infectious Diseases, Tokyo, Japan, were also included. Japanese Ad7 strains 383 (Ad7d) and Bal (Ad7d2) have different BseEI restriction patterns (9, 23).

Clinical records of children hospitalized at the Seoul National University Children’s Hospital were reviewed (11).

Specimens. Nasal aspirate specimens were collected by using mucus traps and catheters. The specimens were refrigerated immediately after collection and processed in the laboratory within 48 h (11, 36).

Viral antigen detection. Indirect immunofluorescence staining for adenovirus was done directly on nasal aspirate specimens by using murine monoclonal antibodies (Chemicon, Temecula, Calif.) and fluorescein isothiocyanate-conjugated rabbit antimurine immunoglobulin G (Cappel, West Chester, Pa.) (11, 36).

Viral culturing and identification. Nasal aspirate specimens were also inoculated into HEp-2, MDCK, and LLC-MK2 culture tubes (11, 36). The culture tubes were observed for 3 to 4 weeks for cytopathic effect or hemadsorption, and viral identification was confirmed by a virus-specific indirect immunofluorescence test.

Serotyping of adenoviruses. Adenovirus serotypes were identified by a neutralization assay with type-specific reference antisera (10, 11, 17, 25). Serotyping was performed to identify the common serotypes in children, Ad1 to Ad7 and Ad3 strains were subsequently propagated on HEp-2 cells in 100-mm petri dishes. Infected HEp-2 cells were incubated at 37°C in 100-mm petri dishes. Infected cells were incubated at 37°C in 5% CO2 for 48 h before processing. Viral DNA was extracted from the infected cell lysates by using a modified Hirt procedure (28).

D NA STRA NCH REAS ON AR NIS. Twelve restriction enzymes that recognized 6 bp were used (BamHI, BclI, BglII, BglIII, BseEI, EcoRI, HindIII, HpaI, SalI, SmaI, XbaI, and XhoI; purchased from KOSCO Central Research Institute, Seoul, Korea, and New England Biolabs, Beverly, Mass.). Aliquots of 0.5 to 1 μg of DNA were digested with 5 to 10 U of each of the above endonucleases according to the manufacturers’ instructions. DNA fragments were separated by electrophoresis in 0.8 or 1.0% agarose gels (Sigma Chemical Co., St. Louis, Mo.), which were prepared and run in 0.5%-concentrated Tris buffer (40 mM Tris-acetate buffer, 2 mM EDTA [pH 8.3]) at 35 V for 8 h. Bands were stained with ethidium bromide (0.1 μg/ml) and visualized by using an image analyzer (Bioimage processing system 3.0; Biometdib Co., Seoul, Korea) or photographed on a transluminator (Scoulun Scientific Co., Ltd., Seoul, Korea) by using a Polaroid camera (Polaroid Corporation, Cambridge, Mass.).

Nomenclature of genome types. Genome type assignments were made by comparing the resulting restriction profiles with the restriction patterns of prototype and other genome types described in the literature and by following the genome type denomination system (8, 18–20, 30).

Phylogenetic analysis. Analysis of genetic relatedness between genome types was performed by using Bio-profile image analysis software (Bio-ID ++, version 99; Vilber Lourmat Biotechnology, Marne la Vallée, France).

RESULTS

Ad3 and Ad7 epidemics. (i) Frequencies of Ad3 and Ad7 in children with LRTIs. Excluding herpes simplex virus, 912 (35.9%) of 2,542 nasal aspirate specimens were positive for a respiratory virus. Two or more viral agents were identified in 30 specimens. Adenoviruses were isolated from 157 children (6.2%), accounting for 17.2% of antigen detection or culture-confirmed diagnoses. Additionally, 110 strains of adenovirus were isolated from respiratory specimens which were obtained from patients with LRTIs and hospitalized in other hospitals and which were referred to the Seoul National University Children’s Hospital for viral diagnosis. In total, 262 adenovirus strains were serotyped; 56 strains (21%) were Ad3 and 98 strains (37%) were Ad7.

(ii) Outbreaks of Ad3 and Ad7. During the observation period, two outbreaks each of Ad3 and Ad7 were identified (Fig. 1 and 2). Ad3 activity was detected from September 1991 to January 1995. Subsequently, there was an explosive epidemic of Ad7 from September 1995 to November 1997 (11). From June 1998 to April 1999, there was another smaller epidemic of Ad7 that was succeeded by an Ad3 epidemic from September 1998 to November 1999.

Identification of genome types by restriction enzyme analysis. Restriction endonuclease fragment profile analysis differentiated seven genome types among 56 Ad3 strains and two genome types among 98 Ad7 strains. Among these, six genome types of Ad3 (Ad3a13, Ad3a14, Ad3a15, Ad3a16, Ad3a17, and Ad3a18) and one genome type of Ad7 (Ad7f) were novel.

(i) Ad3. All 56 Ad3 strains appeared identical to the Ad3a reference strain when digested with BamHI, BclI, BseEI, SalI, EcoRI, and HindIII; but analyses with BglII, BglIII, SmaI, and XbaI distinguished Ad3a from the novel genome types. Digestion patterns with BglII, BglIII, SmaI, and XbaI are shown in Fig. 4. Digestion with BglI yielded three patterns (I to III). Pattern I was identical to that of the previously described Ad3a genome type (19), while the other two patterns (II and III) were novel. Digestion with BglII produced two patterns. Pattern I was identical to that of the Ad3a reference strain (19). Digestion with SmaI revealed three patterns. Pattern I was identical to that of the Ad3a reference strain (19). Patterns II and III were novel. Digestion with XbaI revealed two patterns. Pattern I was identical to that of the Ad3a reference strain (19). Pattern II was identical to that of Ad3d and Ad3-7 (19).

According to the different combinations of fragment patterns obtained with BglI, BglII, SmaI, and XbaI (Table 1), seven different genome types (Ad3a and six other novel genome types) were identified. The novel genome types were designated Ad3a13, Ad3a14, Ad3a15, Ad3a16, Ad3a17, and Ad3a18, in order of isolation, extending the nomenclature of previous studies (8, 19, 30). The Ad3 genome type distribution among the 56 strains were 4 strains of Ad3a, 13 of Ad3a13, 6 of Ad3a14, 5 of Ad3a15, 17 of Ad3a16, 8 of Ad3a17, and 3 of Ad3a18.

(ii) Ad7. In the analysis of restriction endonuclease fragment
profiles for Ad7, only BamHI was able to discriminate two restriction patterns (Fig. 3); the other 11 restriction enzymes failed to reveal different band patterns. One of the patterns was identified as Ad7d. The other, named Ad7l, was a novel genome type.

**Analysis of relatedness among genome types.**

(i) **Ad3.** All of the Ad3 genome types were closely related and shared 91 to 99% pairwise comigrating restriction fragments (PCRFs) (Fig. 4). Ad3a13 was closely related to Ad3a15 and Ad3a16, by 98 and 99% PCRFs, respectively. Ad3a14 and Ad3a17 were also closely related, by 98% PCRFs.

(ii) **Ad7.** Ad7d and Ad7l were closely related, by 98% PCRFs (Fig. 4).

**Temporal distributions of Ad3 and Ad7 genome types.** The temporal distributions of the Ad3 genome types are shown in Fig. 1. Some genome types were identified in both of the Ad3
outbreaks, while others were identified only in one outbreak. In the first Ad3 outbreak, genome types Ad3a, Ad3a13, Ad3a14, and Ad3a15 were isolated. In the second Ad3 outbreak, six genome types, Ad3a, Ad3a13, Ad3a14, Ad3a16, Ad3a17, and Ad3a18, cocirculated. One of the more common genome types (Ad3a15) isolated in the first Ad3 outbreak was not identified in the second outbreak. Some of the genome types (Ad3a, Ad3a13, and Ad3a14) were common in both outbreaks, while most of the strains in the second Ad3 outbreak belonged to genome types (Ad3a16, Ad3a17, and Ad3a18) that were not previously identified.

The temporal distributions of the Ad7 genome types are shown in Fig. 2. A large outbreak of Ad7d began in October 1995, peaked in May to June 1996, and ended at the end of 1996. This outbreak was followed by smaller epidemics of Ad7d and Ad7l in 1998. Ad7l was the predominant genome type during the second outbreak.

Clinical outcomes. The medical records of 28 of 31 children who had Ad3 and 50 of 51 children who had Ad7 and who were admitted to the Seoul National University Children’s Hospital were reviewed. The medical records of four children were not available. The medical records of the 110 children admitted to other hospitals throughout Korea were not reviewed. Twenty-seven of 28 Ad3-infected children recovered, and 1 child infected with Ad3a13 died. The fatality rate for the Ad3 genome type was 3.6%. Forty-one children infected with Ad7 recovered (32 patients with Ad7d and 9 patients with Ad7l), and 9 children died (7 patients with Ad7d and 2 patients with Ad7l). The fatality rate for both Ad7 genome types was 18%.

DISCUSSION

Genome type diversity within an adenovirus serotype is a well-known phenomenon observed in different parts of the world. Many researchers have used a variety of restriction endonucleases and their own nomenclature to classify or denominate the genome types of adenoviruses (1, 8, 19, 30). Therefore, it is confusing to identify and compare genome types of adenoviruses published in the literature, particularly for Ad3, which shows fairly high variability. In the present study, the typing system proposed by Li et al. (19, 20) was used. The standardization of a typing system and a repository of genome types should be established in the future.

All of the Ad3 isolates showed restriction band patterns identical to that of Ad3a with BamHI, BglII, BstEII, EcoRI, HindIII, HpaI, SalI, and XhoI. The combination of restriction fragment patterns produced by SmaI, BglI, BglII, and XbaI yielded seven Ad3a genome types. In contrast to the rather high genetic heterogeneity of Ad3, 98 strains of Ad7 were classified into two genome types by BamHI and the other restriction endonucleases.

Genome types of Ad3 identified in the literature are Ad3p, the prototype strain, and Ad3a through Ad3x, along with their variants (15). The Ad3a variants that have been described are Ad3a1 through Ad3a12 (8, 19, 20, 30). In China, the dominant genome type of Ad3 was Ad3a2, with occasional isolates of Ad3a4, Ad3a5, and Ad3a6, from 1962 to 1988 (20); in Japan, the dominant genome type was Ad3a, with occasional isolates of Ad3a8 and Ad3c, from 1983 to 1991 (12, 21). In this study, we identified six new variants of Ad3a, Ad3a13 through Ad3a18.

In contrast to Ad3 genome types, the worldwide epidemiology of Ad7 is relatively well delineated. Both globally dispersed and geographically restricted Ad7 genome types were identified by restriction analysis, and regional shifts or replacements...
of dominant genome types were documented on different continents.

During the last two decades, changes in the dominant genome types of Ad7 were observed in many parts of the world. In South America, a shift from Ad7c to Ad7h occurred in 1986 (14), and Ad7h subsequently caused serious acute respiratory illnesses in infants and young children in Chile and Argentina (15). In the early 1980s in China, a new genome type, Ad7d (32), replaced Ad7b as the predominant circulating virus. Ad7d2 has emerged as the predominant virus circulating in Israel since 1992 (2). Recent reports demonstrated that Ad7d, Ad7d2, and Ad7h have spread beyond their formerly geographically restricted regions. Ad7d2 and Ad7h have been identified in the United States, where the dominant genome type has been Ad7b (5). Twenty-two Ad7 strains isolated from 1987 to 1992 in Japan were Ad7d (22), and a recent countrywide epidemic of Ad7 in Japan during the period from 1995 to 1998 was attributed to Ad7d2 (23, 35). Ad7h also has been recognized in Japan (9).

In Korea, there was a large epidemic of Ad7d from 1995 to 1997, and it was followed by a smaller epidemic of Ad7d and Ad7l in 1998 to 1999. A change in genome types from Ad7d to Ad7l was observed during these two Ad7 epidemics. It is interesting that Korea had an Ad7d outbreak after two neighboring countries, China and Japan, had Ad7d epidemics (20, 22). It should also be noted that both Korea and Japan recently had Ad7d epidemics during a similar time period; however, the genome types were not identical. The Korean strains of Ad7d and Ad7l and the Japanese strain of Ad7d2 were closely related, with 99% PCRFs for Ad7d and Ad7d2 and 96% PCRFs for Ad7d2 and Ad7l (Fig. 4).

During epidemics, several variant genome types of the same serotype frequently cocirculate, especially when an epidemic involves a large population, and an outbreak in a closed community may be caused by a single genome type (24). Heterogeneity of genome types in a single epidemic is more common for Ad3, as seen in this study, but may also be observed for Ad7 (20, 22). During the epidemic of viral pneumonia in China in 1958, four genome types of Ad7 were isolated from lung specimens obtained at autopsy, and during a 1983 Ad3 outbreak in Beijing, three genome types, Ad3a2, Ad3a4, and Ad3a5, were isolated in 1 month (20). Most of the genome types within a serotype were closely related, suggesting that the variants might have been the result of genetic drift from an epidemic genome type.

Although many studies have described the genetic heterogeneity of adenoviruses, only a few studies have examined changes in genome types in successive outbreaks of Ad3 and Ad7. Mizuta et al. (21) examined Ad3 genome types isolated in successive outbreaks. The Ad3 isolates were mixtures of three genome types, which had all appeared several months before the epidemics. They concluded that the outbreaks were not due to the appearance of a new genome type. However, they used only four restriction endonucleases and therefore encountered limitations in the discrimination of the genome types. In the report of Itakura et al., the genome types of Ad3 isolated during two consecutive epidemics of keratoconjunctivitis were different (12). Itoh et al. suggested that the emergence of new Ad3 genome types may contribute to the replacement of prior adenovirus serotypes associated with conjunctivitis (13). The nationwide epidemic of Ad7 in Japan in 1995 to 1998 occurred with the emergence of Ad7d2 in a setting of low-grade activity of Ad7d during the preceding years (23).

The first nationwide epidemic of Ad7 in Korea described in this study was also associated with the appearance of Ad7d, and the dominant genome type of the subsequent Ad7 epidemic 2 years later was Ad7l. Also, in this study, most of the Ad3 strains in the second epidemic were new genome types. The clinical significance of the emergence of new genome types is not clear at the moment. While some genome types are claimed to be associated with higher virulence (15, 16, 34), differences in fatality rates according to genome types could not be demonstrated in this study. However, the dominance of new genome types in each of the second outbreaks of Ad3 and Ad7 described in this study suggests that changes in genome types may contribute to the occurrence of an epidemic. This suggestion is consistent with the conclusions of Noda et al. (23) but in contrast to the suggestion by Mizuta et al. (21) that outbreaks of Ad3 were due to endemic genome types rather than new types.

In summary, we have described the molecular epidemiology of Ad3 and Ad7 outbreaks in Korea over 11 consecutive years.
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REFERENCES