INFECTION AND IMMUNITY, Nov. 1981, p. 368-372
0019-9567/81/110368-05$02.00/0

Vol. 34, No. 2

Molecular Epidemiology of Adenoviruses: Alternating Appearance of Two Different Genome Types of Adenovirus 7 During Epidemic Outbreaks in Europe from 1958 to 1980

GÖRAN WADELL,[1]* JAN C. DE JONG,[2] AND SIGVARD WOLONTIS[3]

Department of Virology, Umeå University, S-901 85 Umeå, Sweden[1]; The Stockholm County Council Central Microbiological Laboratory, Stockholm, Sweden[3]; and Rijks Instituut vor de Volksgezondheid, Bilthoven, The Netherlands[2]

Received 8 June 1981/Accepted 30 June 1981

Four genome types of adenovirus serotype 7 (Ad7, Ad7a, Ad7b, and Ad7c) were identified by use of DNA restriction enzymes *Bam*HI, *Eco*RI, and *Sma*I. We obtained information on the distribution of the four Ad7 genome types by typing 68 Ad7 isolates obtained in the Netherlands from 1958 to 1979 and 36 Ad7 isolates obtained in Sweden from 1964 to 1980. The Ad7 prototype was never detected, and only seven isolates were genome-typed Ad7a. Ad7b and Ad7c accounted for 94% of the genome-typed isolates obtained from patients. Ad7c was isolated in the Netherlands during 1958 to 1969, but only Ad7b has been detected there since 1970. In Sweden, Ad7c was recovered between 1964 to 1972, but only Ad7b has been isolated there since 1973. In conclusion, the newly recognized Ad7b and Ad7c genome types, which in the Netherlands and Sweden represent virtually all genome-typed isolates from patients, show a mutually exclusive appearance.

Thirty-eight adenovirus serotypes have been recognized up to now (3a, 5, 14). Of these serotypes, adenovirus type 7 (Ad7) is frequently associated with epidemic outbreaks of severe respiratory illness (reviewed in reference 11). In infants, these infections are particularly severe and may be fatal (2, 11). Rowe et al. (6) recognized by serological techniques two subtypes of Ad7 (Ad7 and Ad7a). This distinction has been questioned (13). Distinct entities of viral genomes which are not clearly distinguished by serological methods can be identified by deoxyribonucleic acid (DNA) restriction enzyme analysis. These entities have been designated genome types (9, 11). Using this technique, we originally differentiated three distinct genome types: Ad7 prototype, Ad7a (the vaccine strain), and Ad7b (10).

Analysis of 29 isolates recovered in England from 1973 to 1977 revealed that all were of the Ad7b genome type (11). We have recently found that a large Ad7 epidemic in Sweden during 1964 was caused by a fourth genome type, Ad7c (unpublished data). It was the objective of this study to analyze the distribution of the four Ad7 genome types in Europe. The chronological occurrence of infections caused by different Ad7 genome types was charted by genome typing of 104 Ad7 isolates recovered in Sweden from 1964 to 1980 and in the Netherlands from 1958 to 1980. It was found that the Ad7b and Ad7c genome types, which accounted for 94% of the genome-typed isolates, showed a mutually exclusive appearance.

MATERIALS AND METHODS

Virus strains. The virus strains were recovered from nasopharyngeal swabs and fecal specimens. All isolates reacted as Ad7 in neutralization tests. The Ad7 strains were propagated in A-549 cells, a continuous oat cell carcinoma cell line (provided by W. A. Nelson-Rees, Berkeley, Calif.). Viral DNA was analyzed at the Department of Virology, Karolinska Institutet, Stockholm, Sweden, after one or two passages in tissue culture.

Preparation of viral DNA. Extraction of intracellular viral DNA was performed by a modification of the method described by Hirt, as detailed elsewhere (9).

DNA restriction. The restriction endonuclease *Bam*I was prepared by the method of Bickle et al. (1). *Sma*I was purified in accordance with an unpublished report of a procedure kindly provided by H. J. Monstein, Uppsala, Sweden. *Bam*I was incubated in 10 mM $MgCl_2$-10 mM tris(hydroxymethyl)aminomethane (pH 7.9)-6 mM 2-mercaptoethanol. *Sma*I was incubated in 6 mM $MgCl_2$-20 mM KCl-10 mM tris(hydroxymethyl)aminomethane (pH 9.2)-6 mM 2-mercaptoethanol. All enzyme reactions were carried out for 2 h at 37°C with 1 μg of DNA. The previously described procedure (8) was used with the following modification: 1/10 volume of 37% Ficoll 400 (Pharmacia, Uppsala, Sweden), 50 mM ethylenediaminetetraacetic acid, and 0.3% bromophenol blue were added to ter-

minate the enzyme reaction. The DNA fragments were then electrophoresed on vertical 1.2% (wt/vol) agarose slab gels at 2.3 V/cm^2 at +4, as previously described (9).

RESULTS

Identification of four Ad7 genome types and comparison of their DNA restriction site maps. The DNA cleavage patterns of Ad7, Ad7a, and Ad7b after restriction with *Bam*HI, *Eco*RI, *Hin*dIII, *Hpa*I, and *Sma*I have been previously described (10). A fourth Ad7 genome type, designated Ad7c, was found to have caused the 1964 Ad7 epidemic in Sweden. The Ad7c genome type could be distinguished from the three other Ad7 genome types by its cleavage with *Bam*HI (Fig. 1). The DNA restriction patterns of Ad7b and Ad7c obtained with *Eco*RI, *Hin*dIII, and *Hpa*I were indistinguishable, whereas the pattern of Ad7c obtained with *Sma*I was distinct from those of Ad7 and Ad7b but identical to that of Ad7a. Ad7c could thus be identified either by its cleavage with *Bam*HI or by comparing the cleavage patterns obtained with both *Eco*RI and *Sma*I. The DNA restriction site maps of the four Ad7 genome types obtained with *Bam*HI, *Eco*RI, and *Sma*I are presented in Fig. 2.

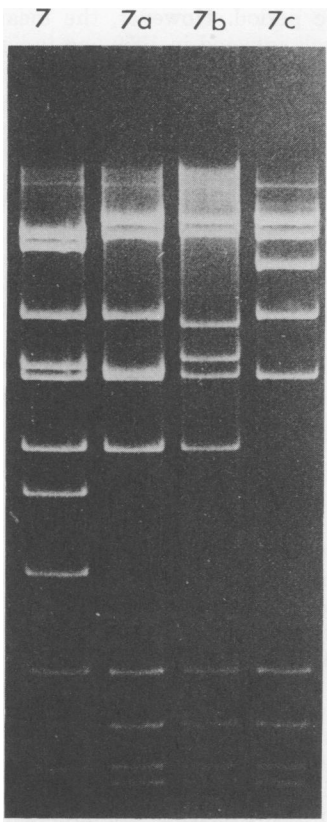

FIG. 1. *DNA restriction patterns after digestion of DNA from the four Ad7 genome types with BamHI. The DNA fragments were separated by electrophoresis in 1.2% agarose slab gels.*

A comparison of the distribution of restriction sites in the four Ad7 genome types and the genome of Ad3 was performed. The degree of homology was estimated in paired comparisons of the number of common restriction sites out of the total number of known restriction sites (Table 1). The degree of homology varied from 73 to 94% among the four Ad7 genome types. Only two restriction sites, positions 85.4 and 92.9, differed between the newly recognized Ad7c genome type and Ad7a (Fig. 2). Of the 32 restriction sites, 28 that mapped in Ad3 (10) and the Ad7c genome type were identical, whereas only 27 of the 37 restriction sites were common to the Ad7 and the Ad7b genome types. This finding reveals that a greater difference in the positions of restriction sites occurred among genome types of serotype 7 than between two different serotypes.

Analysis of the occurrence of the four Ad7 genome types. Adenoviruses have been serotyped since 1958 in Sweden and the Netherlands. It was therefore possible to follow the chronological occurrence of infections caused by the Ad7 genome types in these countries by analyzing Ad7 isolates taken during each year since 1958. In the Netherlands, the Ad7 prototype was never found, and only 7 of the 68 Ad7 isolates were genome-typed as Ad7a. The majority of the isolates were genome-typed as Ad7b and Ad7c. Among these, only Ad7c was detected in isolates taken during 1958 to 1969, whereas Ad7b has been prevalent since 1970 (Fig. 3).

In Sweden, Ad7 isolates could be traced back to 1964 (Fig. 4). DNA restriction analysis of 36 Ad7 isolates obtained during the last 16 years revealed the occurrence of Ad7b and Ad7c genome types only. The Ad7 prototype and the Ad7a genome type were not detected among the Swedish isolates. Ad7c was prevalent in strains obtained from 1964 to 1972, but only Ad7b has been exclusively isolated in Sweden since 1973.

Clinical observations. The principal clinical diagnoses of the 49 Dutch and Swedish patients, who showed seroconversion in complement fixation assays during the period when either Ad7b or Ad7c was isolated, are given in Table 2. The most characteristic symptoms were pneumonia and pyrexia as a single entity or in combination with diarrhea or other symptoms. Five patients had central nervous system disorders ranging

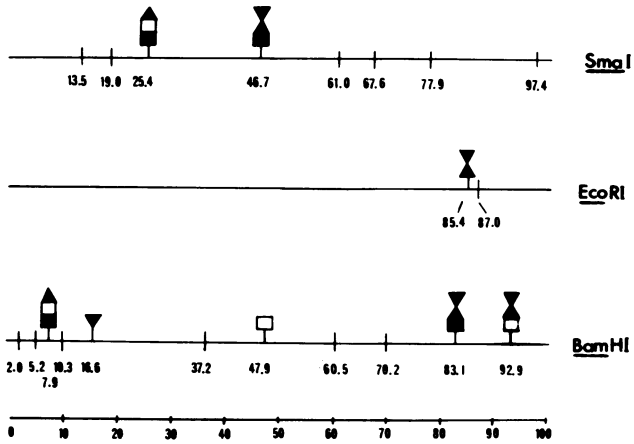

FIG. 2. *Restriction site maps of the four Ad7 genome types obtained with BamHI, EcoRI, and SmaI. The Ad7 (▼), Ad7a (▲), Ad7b (□), and Ad7c (■) genome types are represented. DNA restriction sites which are common to all four genome types are indicated by vertical transverse lines. The restriction sites which are not common to all genome types are indicated by the symbols of the genome type(s) which carries the restriction site.*

TABLE 1. *Paired comparison of the relative numbers of common restriction sites in the genomes of the Ad3 prototype and the four genome types of Ad7*

Pair	Common sites/ total no. of mapped restriction sites	Degree of homology (%)
Ad3/Ad7	31/37	84
Ad3/Ad7a	31/35	89
Ad3/Ad7b	28/36	78
Ad3/Ad7c	28/32	88
Ad7/Ad7a	30/36	83
Ad7/Ad7b	27/37	73
Ad7/Ad7c	27/35	77
Ad7a/Ad7b	29/33	88
Ad7a/Ad7c	30/32	94
Ad7b/Ad7c	28/32	88

from meningeal irritation to encephalitis. No clear-cut difference between the symptoms of patients infected with Ad7b or with Ad7c was noted.

Three of the patients from whom the Ad7a genome type was isolated showed seroconversion in complement fixation assays. The first was a one-year-old with a fever, bronchitis, and meningeal irritation; the second was a four-year-old with abdominal complaints, sore throat, and cough; and the third was a ten-year-old with a fever, headache, and swollen tonsils. The median ages of the Dutch patients from whom Ad7 was recovered was determined for each of the 20 years during which Ad7 was recovered and was found to vary between 2.5 to 4 years. The median age of all patients was 4 years. Only 6 of the 589 patients were older than 13 years.

The interval from 1960 to 1968 was an interepidemic period. However, the mean ages of individuals infected in 1959 and individuals infected in 1969 did not differ significantly.

DISCUSSION

Restriction endonucleases *Bam*HI or *Eco*RI and *Sma*I can be used to define four different genome types of Ad7: Ad7, Ad7a, Ad7b, and Ad7c. Ad7 is the adenovirus type which in the Netherlands is most frequently associated with disease. During the period 1958 to 1973, 26% of 1798 adenovirus isolates were serotyped as Ad7 (3).

The chronological occurrence of the four Ad7 genome types was determined during the epidemics of 1959 (Ad7c), 1969 (Ad7b), 1973 (Ad7b), and 1978 (Ad7b) in the Netherlands. The 1959 epidemic coincided with an Ad7c epidemic in Sweden. Furthermore, the 1973 and 1978 epidemics in the Netherlands coincided with epidemics of Ad7b in England (8, 12).

The mean age of the Ad7-infected Dutch children was calculated to find a clue to the interepidemic interval in the Netherlands. Since the infected children at the end of the 1959 epidemic were of the same age as the children infected in 1969, it was considered likely that silent infections of less virulent Ad7 strains could have immunized the Dutch children during the interepidemic interval. This suggestion is difficult to prove or disprove, since the immunological status of Dutch children at the time of reappearance of major Ad7 epidemics is not known.

Altogether, we genome-typed 176 Ad7 isolates in the present and past studies (10, 11; Wadell et al., submitted for publication). Among Ad7 iso-

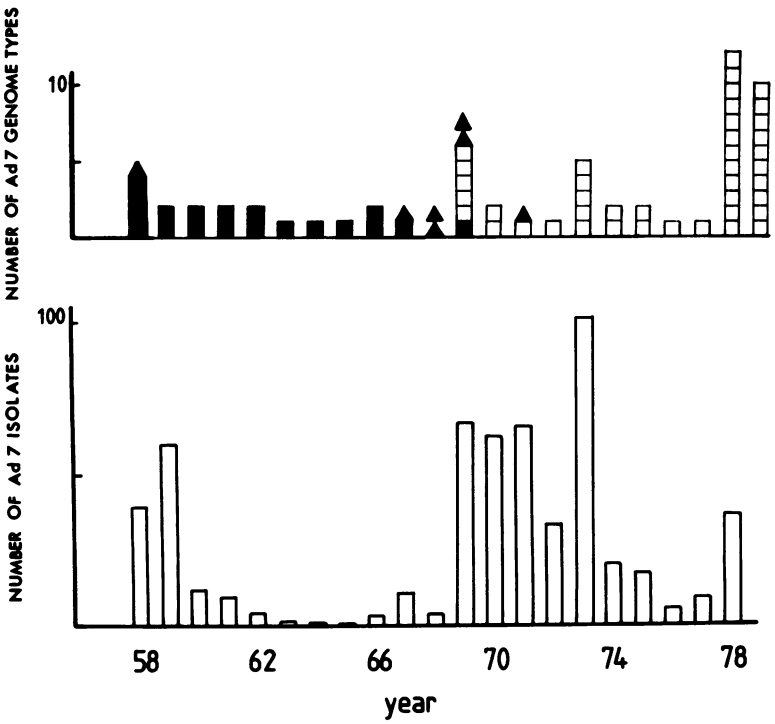

FIG. 3. *Number of Ad7 isolations obtained in the Netherlands from 1958 to 1978 (lower half) and distribution of the genome-typed Ad7 isolates (upper half). Ad7a, Ad7b, and Ad7c are represented by the symbols given in the legend to Fig. 2.*

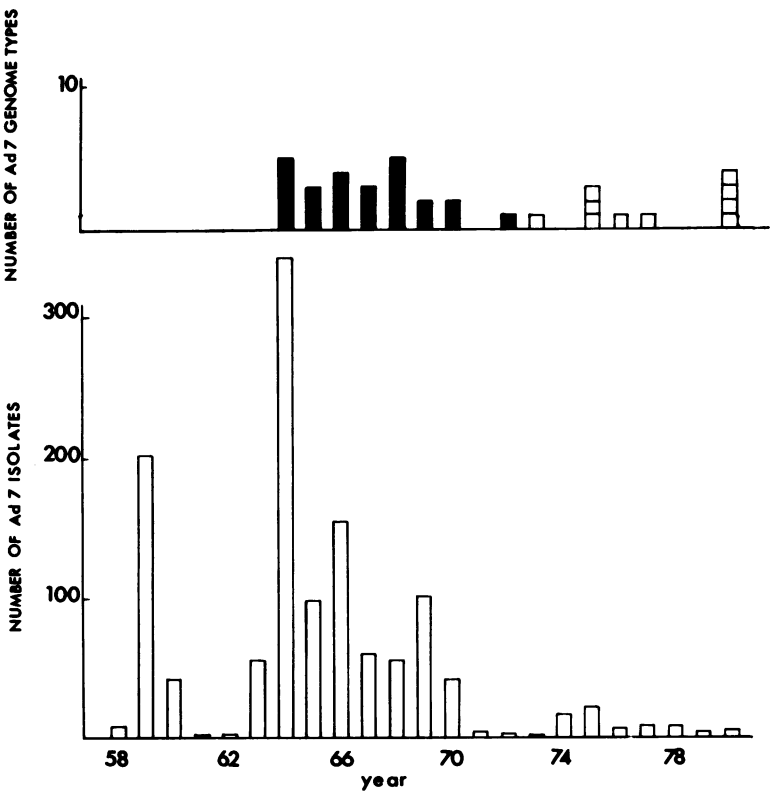

FIG. 4. *Number of Ad7 isolations obtained in Sweden from 1958 to 1979 (lower half) and distribution of the genome-typed Ad7 isolates (upper half). Ad7b and Ad7c are represented by the symbols given in the legend to Fig. 2.*

TABLE 2. *Clinical diagnoses of Dutch and Swedish patients from whom the Ad7b or Ad7c genome type was isolated*

Clinical diagnosis	No. of patients	
	Ad7b	Ad7c
Pyrexia of unknown origin	13	3
Central nervous system disorders	2	3
Conjunctivitis	1	
Upper respiratory tract infection	1	
Pharyngitis	1	1
Lower respiratory tract infection[a]	8	4
Diarrhea and pyrexia	2	3
Diarrhea and lower respiratory tract infection	2	5

[a] Pneumonia or pseudocroup: one case.

lates from patients with clinical symptoms, 2 isolates of the Ad7 genome type, 7 isolates of Ad7a, and 159 isolates of Ad7b or Ad7c genome type were detected.

Genome typing of the Ad7 isolates obtained during the Seattle Virus Watch program (4) showed that Ad7, Ad7a, and Ad7b were prevalent during the same period (Wadell et al., submitted for publication). The mutual exclusion of Ad7b and Ad7c genome types during 16 years in Sweden and 21 years in the Netherlands is therefore intriguing.

The observation that the Ad7c genome type was responsible for the severe Ad7 infections in the 1960s and was then succeeded by the Ad7b genome type in the 1970s is not limited to Sweden and the Netherlands. Ad7 isolates obtained during a 1964 epidemic outbreak in Norway were genome-typed as Ad7c, and 29 Ad7 wild-type isolates obtained during 1973 and 1977 in England were of the Ad7b genome type (12). In addition, the outbreak of respiratory disease in Lower Saxony, West Germany, during 1979 (12) was caused by Ad7 strains, five of which were genome-typed as Ad7b.

The Ad7b genome type was identified (11) as the cause of the severe Ad7 epidemic in Paris during 1956 (2). This genome type has thus not emerged for the first time. It is therefore possible that the Ad7b and the Ad7c genome types alternate over the decades as the etiological agents of the epidemic outbreaks caused by Ad7.

ACKNOWLEDGMENTS

Gunnar Sundell and Ingrid Rehnlund provided excellent technical assistance.

This study was supported by grants from the Swedish Medical Research Council (B81-16X-05688) and the Swedish Society of Medical Science.

LITERATURE CITED

1. **Bickle, T. A., V. Pirotta, and R. Imber.** 1977. A simple procedure for purifying restriction endonucleases. Nucleic Acids Res. **4**:2561-2572.
2. **Chany, C., P. Lepine, L.-T. Vinh, P. Satge, and J. Virar.** 1958. Severe and fatal pneumonia in infants and young children associated with adenovirus infections. Am. J. Hyg. **67**:367-378.
3. **de Jong, J. C., and C. J. Muzerie.** 1973. Adenovirus infecties. Berichten Rijks. Inst. Volksgezondheid Utrecht, p. 162-166.

3a. **de Jong, J. C., R. Wigand, G. Wadell, D. Keller, C. J. Muzerie, A. G. Wermenbol, and G. J. P. Schaap.** 1981. Adenovirus 37: identification and characterization of a medically important new adenovirus type of subgroup D. J. Med. Virol. **7**:105-118.

R 4. **Fox, J. P., C. E. Hall, and M. K. Cooney.** 1977. The Seattle virus watch. VII. Observations on adenovirus infections. Am. J. Epidemiol. **105**:362-386.

5. **Johansson, M., I. Uhnoo, A. Kidd, C. R. Madeley, and G. Wadell.** 1980. Direct identification of enteric adenovirus, a candidate new serotype, associated with infantile gastroenteritis. J. Clin. Microbiol. **12**:95-100.
6. **Rowe, W. P., J. W. Hartley, and R. J. Huebner.** 1958. Serotype composition of the adenovirus group. Proc. Soc. Exp. Biol. Med. **97**:465-470.
7. **Sutton, R. N. P., H. J. M. Pullen, P. Blackledge, E. H. Brown, L. Sinclaire, and P. N. Swift.** 1976. Adenovirus type 7; 1971-1974. Lancet **ii**:987-991.
8. **Varsànyi, T., G. Winberg, and G. Wadell.** 1977. DNA restriction site mapping of adenovirus type 16 with BamI, EcoRI, HpaI, and SalI. FEBS Lett. **76**:151-158.
9. **Wadell, G., and J. C. de Jong.** 1980. Use of restriction endonucleases for identification of a genome type of adenovirus 19 associated with keratoconjunctivitis. Infect. Immun. **27**:292-296.
10. **Wadell, G., and T. M. Varsànyi.** 1978. Demonstration of three different subtypes of adenovirus type 7 by DNA restriction site mapping. Infect. Immun. **21**:238-246.
11. **Wadell, G., T. M. Varsànyi, A. Lord, and R. N. P. Sutton.** 1980. Epidemic outbreaks of adenovirus type 7 with special reference to the pathogenicity of adenovirus genome type 7b. Am. J. Epidemiol. **112**:619-628.
12. **World Health Organization.** 1979. Virus diseases surveillance. Adenovirus type 7. WHO Weekly Epidemiol. Rec. **54**:279-280.
13. **Wigand, R.** 1976. Does adenovirus subtype 7a exist? Arch. Virol. **50**:335-337.
14. **Wigand, R., H. Gelderblom, and G. Wadell.** 1980. New human adenovirus (candidate adenovirus 36), a novel member of subgroup D. Arch. Virol. **64**:225-233.