Molecular epidemiology of ocular isolates of adenovirus 8 obtained over nine years

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Abstract

Twenty nine strains of adenovirus 8 have been isolated over nine years in Strasbourg, France, 22 of which were from one private ophthalmologist. To assess a possible relation between these strains, the DNA of adenovirus was analysed by restriction fragment length polymorphism using eight different enzymes. Among these, three proved discriminant (Xba I, Bgl II, Eco RI) and made it possible to define 13 genotypes differing from each other by one to three DNA bands. Seven genotypes were unique isolates, while three, representing 16 strains, were isolated over five to eight years. All the genotypes but one were closely related, with 87% homology. All 13 differed from an adenovirus 8 strain from Lyon (homology 68–76%). This study confirmed the stability of adenovirus 8 in a given population.

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Adenovirus 8 is a common cause of epidemic keratoconjunctivitis that may be transmitted by ocular examination apparatus. These infections are without any long term consequences on eye function. The epidemiology of adenoviruses is currently under investigation using restriction fragment length polymorphism (RFLP) of the entire adenovirus genome. This allows identification of genomic variants (genotypes) and analysis of their distribution in time and space.[1–5]

Methods

From 1989 to 1997, 29 strains of adenovirus 8 have been isolated in the virology laboratory, Faculty of Medicine, Strasbourg, France. Twenty two strains were from one private ophthalmologist, three from another private specialist, and four from different units of the Strasbourg University Hospital.

All the strains were isolated on human diploid fibroblasts, MRC-5, serotyped by seroneutralisation or immunofluorescence with a monoclonal antibody at the time of isolation, and stored at −80°C. For RFLP analysis, viruses were grown on MRC-5 cells. The intracellular viral DNA was extracted according to the method of Shinagawa *et al*[6] modified by Li *et al*.[7] Briefly, infected cells were recovered in a 50 ml tube, pelleted (1500 *g*, 15 minutes, 4°C), and suspended in 1.5 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.9). Cells were lysed by 0.01% sodium dodecyl sulphate (SDS) in 1 M NaCl overnight at 4°C. The cellular debris was pelleted (17 500 *g*, 30 minutes, 4°C) and the virus containing supernatant was extracted with an equal volume of phenol. The phenolic phase was extracted twice with equal volumes of TE buffer, after which 1.5 volumes of 95% ethanol were added to the aqueous phase and left overnight at −20°C. After centrifugation (1500 *g*, 15 minutes, 4°C) the pellet was suspended in 500 µl of lysis buffer (0.5% SDS, 100 mM NaCl, 50 µg/ml proteinase K) and incubated at 37°C for about one hour. After two successive extractions with equal volumes of phenol and ether, the aqueous phase was incubated with an equal volume of isopropanol overnight at −20°C. After centrifugation (17 000 *g*, 20 minutes, 4°C), the pellet of purified adenovirus DNA was dissolved in 100 µl of TE buffer. DNA concentrations were estimated spectrophotometrically.

Four micrograms of DNA were digested by each of the eight hexameric restriction enzymes (BamHI, BglII, EcoRI, HindIII, PstI, SalI, SmaI, XbaI) according to manufacturer's recommendations. The digests were submitted to electrophoresis in immersed ethidium bromide containing (0.5 µg/ml) agarose gel (0.8% wt/vol) in TBE buffer (89 mM Tris-borate, 2.5 mM EDTA) under 6 V/cm. DNA bands were visualised on an ultraviolet light transilluminator, and restriction patterns compared using a molecular weight marker (EcoRI-HindIII digested DNA). Each DNA digestion and electrophoresis was repeated three times. Genomic homology between two strains was calculated using the percentage of pairwise comigrating restriction fragments (% PCRF) which corresponds to the comigrating restriction fragments of a pair divided by the total number of bands of the pair.[7] A numerical code for each restriction profile was attributed in the chronological order of strain isolation to separate genomic variants. A non-epidemiologically related adenovirus 8 strain (kindly provided by Professor Aymard, Faculté de Médecine, Lyon, France) was included in the analysis as an outgroup.

Results

Five enzymes (BamHI, HindIII, PstI, SalI, SmaI) gave identical profiles with the 29 strains and were not further considered. XbaI was the most discriminating restriction enzyme and separated the strains into four profiles (six to nine fragments; 85–96% PCRF). BglII gave three different patterns (five to eight 8 fragments; 96–98% PCRF), and EcoRI only two (four or five fragments; 97–98% PCRF).

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Table 1 Per cent pairwise comigrating restriction fragments (PCRF)

Year	No	GT	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	Lyon
93	1	I		86	88	84	82	85	84	86	86	85	84	82	84	65
94	1	II			98	95	97	93	95	93	91	87	93	95	93	76
91	1	III				97	95	95	93	92	90	89	91	93	95	74
89–96	6	IV					98	97	96	95	92	91	93	95	97	70
91–97	5	V						96	98	96	93	89	95	97	95	72
90–93	5	VI							98	96	93	92	95	93	95	71
91	2	VII								98	95	91	97	95	93	74
95	1	VIII									97	93	95	93	92	74
90	2	IX										96	98	96	95	72
90	1	X											94	92	94	69
91	1	XI												98	96	72
91–93	2	XII													98	70
93	1	XIII														68

GT, genotype.

The combination of the profiles provided by these three enzymes allowed us to separate the strains into 13 genotypes which differed by one to three bands (PCRF ranging from 83% to 98%). All these genotypes clearly differed from the Lyon strain with a percentage PCRF ranging between 65% and 76% (table 1).

Seven genotypes were found to be unique isolates. On the other hand, genotype IV was found six times over seven years, genotype V, five times over six years, and genotype VI, five times over four years (table 2), all three being closely related (96–98% PCRF). All isolates of these three genotypes were from the same private ophthalmologist, save for two from another private specialist (genotype IV, 1996; and genotype VI, 1993) and one from a hospital unit (genotype V, 1994). The third isolate from the second private was of genotype XIII and the three additional hospital isolates were of genotypes II (one strain) and IX (two strains). Genotype I differed from all the others, with a PCRF of 82–86%. Thus no clear relations could be established between genotype and strain origin.

Discussion

Genome stability of adenovirus 8 has already been described by Kemp and Hierholzer[8] and Adrian *et al*,[9] using only five and six restriction enzymes, respectively. However, it is difficult to compare studies for which different enzymes have been used. Different results have been obtained with the same enzyme according to the region which strains originated from. For example, HindIII was reported as the most discriminating restriction enzyme in the studies of Kemp and Hierholzer[8] and Adrian *et al*,[9] while it gave no difference between strains for de Jong *et al*[2] or in our study. The most discriminating enzyme for our strains, XbaI, has not been used by the other investigators.

de Jong *et al* showed that different genotypes of adenovirus 8 circulated in the restricted area of Brest, France, over a six year survey and were associated with consecutive epidemic keratoconjunctivitis epidemics.[2] We have found that closely related genomic variants of adenovirus 8 were in circulation in the Strasbourg population during nine years. Unfortunately, we had only limited information about the patients, so it is difficult to establish the affiliation between these identical strains. However, contamination from a common source is unlikely because the private ophthalmologist was clearly aware of adenovirus infections and regularly sent samples to the virology laboratory; in addition the same genotypes were found in different locations. The repeated isolation of closely related genotypes reinforced the notion of stability of the adenovirus genome and the limited differences between most genotypes are in favour of a common ancestor. RFLP analysis only delineates nucleotide positions and may not represent the true variability of the genome. However, it proves discriminant enough to warrant its use for the epidemiological analysis for adenovirus strains belonging to the same serotype. For example, adenoviruses are increasingly responsible for infections with a fatal outcome in immunocompromised patients for which a nosocomial origin must be ruled out.[10] Thus it might be of interest to devise a consensus panel of enzymes for such studies.

Table 2 Genotypes (number of strains) of adenovirus 8 isolated in Strasbourg over nine years

1989	1990	1991	1992	1993	1994	1995	1996	1997
IV (2)	VI (1) IX (2) X (1)	III (1) IV (1) V (1) VI (3) VII (2) XI (1) XII (1)		I (1) IV (1) V (1) VI (1) XII (1) XIII (1)	II (1) V(2)	IV (1) VIII (1)	IV (1)	V (1)

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1 Adrian T, Best B, Hierholzer JC, *et al.* Molecular epidemiology and restriction site mapping of Adenovirus type 3 genome types. *J Clin Microbiol* 1989;**27**:1329–34.
2 de Jong JC, Démazure M, Legrang-Quillen MC, *et al.* New developments in the molecular epidemiology of adenovirus 8 keratoconjunctivitis. *J Med Virol* 1992;**38**:102–7.
3 Li Q-G, and Wadell G. Analysis of 15 different genome types of adenovirus type 7 isolated on five continents. *J Virol* 1986;**60**:331–5.
4 Li QG, Wadell G. Comparison of 17 genome types of adenovirus type 3 identified among strains recovered from six continents. *J Clin Microbiol* 1988;**26**:1009–15.
5 Li QG, Zheng QJ, Liu YH, *et al.* Molecular epidemiology of adenovirus types 3 and 7 isolated from children with pneumonia in Beijing. *J Med Virol* 1996;**49**:170–7.
6 Shinagawa M, Matsuda A, Ishiyama T, *et al.* A rapid and simple method for preparation of adenovirus DNA from infected cells. *Microbiol Immunol* 1983;**27**:817–22.
7 Li QG, Hambraeus J, Wadell G. Genetic relationship between thirteen genome types of Adenovirus 11, 34, and 35 with different tropisms. *Intervirology* 1991;**32**:338–50.
8 Kemp MC, Hierholzer JC. Three adenovirus type 8 genome types defined by restriction enzyme analysis: prototype stability in geographically separated populations. *J Clin Microbiol* 1986;**23**:469–74.
9 Adrian T, Wolf U, Lauer HJ, *et al.* Restriction site mapping of adenovirus type 8 genome types. *Res Virol* 1990;**141**:611–62.
10 Hierholzer JC. Adenoviruses in the immunocompromised host. *Clin Microbiol Rev* 1992;**5**:262–74.