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

D Corsaro, J P Gut, V Venard, A Le Faou

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.

Keywords: adenovirus 8; molecular epidemiology; eye isolates

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.

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 were not further considered. XbaI was the most discriminating restriction enzyme and was not further considered. XbaI was the most discriminating restriction enzyme and was not further considered. XbaI was the most discriminating restriction enzyme and was not further considered.

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 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. 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 fragments; 96–98% PCRF), and EcoRI only two (four or five fragments; 97–98% PCRF).
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 Hierholzer4 and Adrian et al.,3 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 Hierholzer4 and Adrian et al.,3 while it gave no difference between strains for de Jong et al20 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 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

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GT, genotype.

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