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AN OUTBREAK OF RESPIRATORY DISEASE DUE TO A TYPE 5 ADENOVIRUS IDENTIFIED AS GENOME TYPE 5a

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Summary. — A cluster of adenovirus type 5-caused acute respiratory diseases was observed in Prague in the autumn-winter season of 1978/79. An analysis of viral DNA by sequence-specific restriction enzymes in two out of 39 isolates revealed that these isolates, identical to each other, were clearly distinct from adenovirus type 5 prototype strain. The designation "adenovirus genome type 5a" is suggested for these isolates.

Key words: respiratory virus watch programme; adenovirus genome type 5a; genome typing; DNA specific restriction enzymes

Introduction

Within a respiratory virus watch programme carried out in Prague since 1962, a cluster of adenovirus type 5-caused acute respiratory diseases was observed in the autumn-winter season of 1978/79. An unusally high isolation rate, a fast but time-limited spread of these adenoviruses among the population and a relatively high percentage of lower respiratory tract involvement suggested some distinction of these isolates from previously circulating adenovirus type 5 (Ad 5) strains. Therefore a detailed analysis of the viral genome of two of the Ad 5 isolates was performed in comparison with the prototype Ad 5 strain.

Some of the epidemiological, clinical and genometyping findings are presented below.

Materials and Methods

Clinical material. The virological examination was performed on 4 groups of Prague inhabitants: *a*) children aged 0—14 years, hospitalized at the IIIrd Child Clinic in Prague 2; *b*) children aged 0—14 years treated for acute respiratory disease (ARD) as outpatients at Child Health Centre in Prague 3; *c*) apprentices of a trainess' centre of a Prague factory (aged 15—19 years) treated for ARD as outpatients; and *d*) students aged 18—25 years, living on a Prague college campus, treated for ARD as outpatients. The materials were collected at the acute stage of the illness (up to the 5th day after onset) during a period of seasonal occurrence of respiratory diseases from October to May. Normal persons were not examined.

Virus isolation and identification. Nasopharyngeal swabs or washings taken at the acute stage of the disease were inoculated into embryonated eggs, primary monkey kidney cells, diploid cells

Table 1. Distribution of Ad 5 isolates in the course of the 1978/1979 season

Month	No. of ARD cases examined	No. of Ad 5 isolates
October, 1978	56	5
November, 1978	67	26
December, 1978	50	4
January, 1979	44	0
February, 1979	68	0
March, 1979	69	2
April, 1979	27	1
May, 1979	32	1
Total	413	39

and several continuous cell lines (HEp-2, HeLa, L-132) to isolate a broad spectrum of respiratory viruses. Adenovirus isolates were identified in complement fixation (CF) tests with adenovirus group-specific antiserum and typed in virus neutralization (VN) tests with type-specific horse antisera (CDC, Atlanta, U.S.A.).

Serology. Where possible, acute and convalescent sera were obtained and titres of specific antibodies in paired sera were determined. A group-specific adenovirus antigen was used in CF tests and a fresh Ad 5 isolate in VN tests.

Characteristics of strains selected for analysis of the viral genome. Strain No. 3315/78 (throat swab) was isolated in November, 1978 from a hospitalized child aged 9 years with bronchopneumonia. The patients' acute and convalescent serum had titres of 8 and 16 (CF test) and 40 and 40 (VN test), respectively. Strain No. 3349/78 (throat swab) was isolated in November, 1978 from a hospitalized child aged 18 months with bronchitis spastica. The patients' acute and convalescent serum had titres of 8 and 32 (CF test) and 160 and 320 (VN test), respectively.

Analysis of viral DNA by sequence-specific restriction enzymes. Viral DNA was prepared, restricted and analysed by agarose slab gel electrophoresis as described (Varsanyi *et al.*, 1977; Wadel and Varsanyi 1978). DNA from the Ad 5 isolates was restricted by BamHI, E. coRI, H. paI, HindIII, KpnI, SalI, SmaI and XhoI. BamHI was purified according to Bickle *et al.* (1977) and SmaI was purified according to an unpublished procedure kindly provided by H. J. Monstein, Uppsala. The remaining enzymes were purchased from New England Biolabs. The enzyme reactions were performed according to the procedures described in the New England Biolabs Catalogue. Ad 7b DNA cleaved with BamHI was used as a molecular weight reference; BamHI cleaved the Ad 7b genome into 10 fragments: 6.25, 5.27, 2.92, 2.48, 2.25, 1.67, 0.67, 0.54, 0.46 and 0.43×10^6 (Wadell and Varsanyi, 1978). DNA fragments smaller than 1% of the unit length DNA are not resolved by the techniques used.

Results

The total numbers of ARD cases over the 1978/79 season and of Ad 5 isolates are shown in Table 1. A cluster of Ad 5-caused ARD occurred in November, 1978. The Ad 5 strains isolated represented 39% of all ARD cases examined in that month.

An analysis of the occurrence of adenovirus serotypes in Czechoslovakia since 1970 revealed that the highest number of Ad 5 strains was isolated in 1978. In that year Ad 5 accounted for 42.5% of all the adenovirus isolates. The mean Ad 5/Ad total rate for 1970—1977 was 6.5% (Table 2).

Table 2. Ad 5 occurrence in Czechoslovakia in 1970—1978

Year	Total No. adenovirus strains isolated	Ad 5 strains isolated	
		No.	%
1970	38	1	2.6
1071	74	4	5.4
1972	73	4	5.5
1973	70	5	7.1
1974	81	5	6.2
1075	62	4	6.4
1976	85	9	10.6
1977	38	3	7.9
1978	94	40	42.5

The Ad 5 strains were isolated mostly in the first passage, 4 to 8 days after inoculation, in at least two different continuous cell lines used for primary isolation. Infectious material was maintained unfrozen for up to 4—5 hr, inoculated in cell cultures and then stored at −60 °C. Ad 5 strains were isolated equally well from both frozen and unfrozen specimens.

Paired sera were obtained from 18 Ad 5-yielding patients. The VN test revealed a fourfold increase in specific antibody in 8 patients; titres of $\geqq 20$

Table 3. Restriction fragments of Ad 5 prototype (Ad 5) and Ad 5a DNA

Fragment	Mol. wt. × 10^{-6} of fragments*					
	BamHI		HindIII		HpaI	
	Ad 5	Ad 5a	Ad 5	Ad 5a	Ad 5	Ad 5a
A	13.7	9.3	5.40	5.40	6.70	6.70
B	9.3	6.7	3.55	3.55	6.45	6.45
C		4.0	3.35	3.35	5.05	5.05
D		3.0	2.95	2.25	2.55	2.15
E			2.25	2.15	0.92	0.92
F			1.89	1.89	0.82	0.82
T			1.84	1.84	0.60	0.60
H			1.33	1.33		
I			0.62	0.79		
J				0.62		
Sum of fragments	2	4	9	10	7	7
Fragments comigrating	1		8		6	

* Obtained by comparison to the migration of Ad 7b BamHI fragments. For restriction fragments of high mol. wt. the information on the Ad 5 prototype (compiled by Dr. Vivien Mautner and distributed at the EMBO Workshop on the Molecular Biology of Adenoviruses held at Orenäs, Sweden, June 11—16, 1978) was used.

in both serum samples were recorded in 6 and titres of < 10 in 4 patients. In the CF test, an increase in adenovirus antibodies was found in 4 cases; a titre of $\geqq 16$ in both serum samples was found 12 times and a titre of < 8 in both samples twice.

As to the clinical manifestations of Ad 5 infection in 39 patients, the lower respiratory tract was involved, mostly in the form of bronchopneumonia, in 11 (28%) cases. The mean age of patients with lower respiratory tract was almost 3 times lower (4.5 years) than that of patients with affections of the upper respiratory tract (12.6 years).

The restriction analysis of the two actual Ad 5 isolates and the Ad 5 prototype with the sequence-specific nucleases E. coRI, KpnI, SalI, SmaI and XhoI revealed identical restriction patterns. However, restriction with BamHI, HpaI and HindIII demostrated that the Ad 5 prototype was clearly distinct from the two Ad 5 isolates from the Czech outbreak (Fig. 1, Plate XIII). These are subsequently designated Ad 5a. The Ad 5a genome contained two BamHI restriction sites within the Ad 5 prototype BamHI A fragment.

The restriction patterns of the Ad 5 prototype and the Ad 5a genomes obtained with HindIII corresponded to each other with the exception that the Ad 5a genome contained a HindIII restriction site in the Ad 5 prototype D fragment, yielding the Ad 5a HindIII fragments E and J (Table 3).

Also the HpaI restriction patterns of the Ad 5 prototype and the Ad 5a corresponded to each other with the exception that the Ad 5 prototype HindIII D fragment most likely corresponded to the Ad 5a HindIII D fragment in addition to fragments with a low mol. wt. which were not resolved under the electrophoresis conditions used (Fig. 1, Table 3).

Discussion

An analysis of the occurrence of adenovirus types in Czechoslovakia since 1970 showed a predominance of Ad 5 in 1978 that almost 7 times exceeded the mean occurrence of Ad 5 in the years 1970—1977 (Brůčková *et al.*, 1976; Syrůček *et al.*, 1979).

The mean share of adenoviruses (without type distinction) in the aetiology of ARD reached about 6% in our studies in 1962—1977 (Soběslavský *et al.*, 1970; Syrůček *et al.*, 1979). These findings compare well with those of Foy and Graystone (1976), who reported that, especially in children up to 5 years of age, about 5% of all lower respiratory tract diseases tend to be associated with adenoviruses. Also Brandt *et al.*, (1969) claim that adenoviruses caused 2—7% of lower respiratory tract disease, Ad 5 amounting to 11% of all isolated adenovirus strains.

The results of our virus watch programme in the autumn-winter season of 1978/79 differed from the preceding years by a generally higher share of adenoviruses in the aetiology of ARD (12% as against 6%) and by an enormously high number of Ad 5 isolates, especially at the time of the November 1978 cluster, when Ad 5 caused 39% of all the ARD cases examined. The aetiological relation to the disease was confirmed not only by isolation of Ad 5 from the patients' nasopharynges but also by seroconversion.

The tendency of Ad 5 to appear in a time cluster has been pointed out by Kloene *et al.* (1970).

In 28% of the Ad 5-positive cases examined the infection was manifested as a lower respiratory tract disease occurring at a mean age of 4.5 years. In our previous studies, Ad 5 caused affection of the lower respiratory tract in children at a rate of 18% only (Brůčková *et al.*, 1976).

The unusually high occurrence of Ad 5 in the present study as well as the epidemiological and clinical peculiarities of the disease caused by it called for a detailed analysis of the viral genome. This analysis, performed by DNA sequence-specific restriction enzymes, showed that the two Ad 5 1978/79 isolates examined were identical with each other and also with an Ad 5 isolate described by Faulkner and van Royen (1962) that had been obtained from the cerebrospinal fluid, but clearly distinct from the Ad 5 prototype strain.

It may be concluded that the causative agent of the outbreak in Prague in the 1978/79 season was a type 5 adenovirus distinct from the established prototype strain and which would deserve a distinctive designation. As such, adenovirus genome type 5a is proposed.

References

Bickle, T. A., Pirotta, V., and Imber, R. (1977): A simple general procedure for purifying restriction endonucleases. *Nucleic Acids Res.* **4**, 2561–2572.

Brandt, C. D., Kim, H. W., Vargosko, A. J., Jeffries, B. C., Arrobio, J. O., Rindge, B., Parrott, R. H., and Chanock, R. M. (1969): Infections in 18,000 infants and children in a controlled study of respiratory tract disease. I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. *Am. J. Epidem.* **90**, 484–500.

Brůčková, M., Kunzová, L., Berkovičová, V., and Syrůček, L. (1976): The occurence of different types of adenoviruses in ČSSR in 1970–1974. *Acta hyg., epidem. microbiol.* **1**, 75–84.

Faulkner, R., and van Rooyen, L. (1962): Adenoviruses types 3 and 5 isolated from the cerebrospinal fluid of children. *Canad. med. Ass. J.* **87**, 1123–1125.

Foy, H. M., and Grayston, J. T. (1976): Adenoviruses., pp. 56–57. In A. S. Evans (Ed.): *Viral infections of humans*, Plenum Medical Book Co., New York.

Kloene, W., Bang, F. B., Chakreborty, S. M., Cooper, M. R., Kullemann, H., Ota, M., and Shah, K. V. (1970): A two-year respiratory virus survey in four villages in West Bengal, India. *Am. J. Epidem.* **92**, 307–320.

Soběslavský, O., Brůčková, M., Kunzová, L., Vojtěchovský, K., and Syrůček, L. (1970): Adenovirus, RS virus and *M. pneumoniae* infections in young population of Prague in 1962–1967. *J. Hyg. Epidem., Microbiol. Immunol.* **14**, 350–359.

Syrůček, L., Brůčková, M., and Kunzová L. (1979): Adenovirové infekce v ČSR v letech 1976 – 1979. *Čsl. Epidem. Mikrobiol. Immunol.*, in press.

Varsanyi, T., Winberg, G., and Wadell, G. (1977): DNA restriction site mapping of adenovirus type 16 with Bam I, Eco Ri, Hpa I and Sal. I. *FEBS Lett.* **76**, 151–158.

Wadell, G., and Varsanyi, T. M. (1978): Demonstration of three different subtypes of adenovirus type 7b DNA restriction site mapping. *Infect. Immun.* **21**, 238–246.